ACTA RADIOLOGICA

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OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF DENMARK, FINLAND, NORWAY AND SWEDEN

THERAPY PHYSICS BIOLOGY

INDICES to Vol. 13 (1974)

February April June August October December

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The present investigation suggests that lumbar myelography with methiodal (Kontrast U) after preceding spinal anesthesia is more likely to produce adhesive arachnoiditis than myelography with methylglucamine iothalamate (Conray 60) or its dimer (Dimer X) Furthermore, it appears that operation per se for herniation of the affected disc involves a definite risk of adhesive arachnoiditis irrespective of the type of contrast medium used in the prior myelography

KIM Y W DEBOER UNGER JUNE and GRINSELL P J Post-operative pseudodiverticula (spurious meningoceles) of the cervical subarachnoid space Acta radiol Diagnosis 15 (1974) 16

Nine cases of pseudodiverticula of the cervical subarachnoid space following laminectomy and dentate section are described Three patients within the group developed unexpected symptoms post-operatively presumed related to the pseudodiverticula Complete disappearance of the structures was observed within six months of the operative procedure

RAPOPORT S I, THOMPSON H K and BIDINGER JEANNETTE M Equi-osmolal opening of the blood brain barrier in the rabbit by different contrast media Acta radiol Diagnosis 15 (1974) 21

Six contrast media of differing composition when perfused into the internal carotid artery of rabbits for 20 seconds opened the blood brain barrier at a mean osmotic threshold of 1 16±0 27 SD osmolal, not different from the 1 26 threshold osmolality for NaCl The approximately equi osmolal thresholds for barrier opening by the media and NaCl suggests that the contrast media open the tight junctions between cerebrovascular endothelial cells, probably by osmotically shrinking the cells themselves

KOVÁCS A Observation of the cervical segment of the spinal canal by an extension device Acta radiol Diagnosis 15 (1974), 33

For the diagnosis of cervical ligament and dura thickenings as well as discogenic myelopathies myelography is a generally adopted method In early cases patients are not willing to give their consent to this examination while in late cases the result of operation is rarely satisfactory By a special device exerting traction on the head and bringing the neck in ante flexion the number of cases diagnosed on conventional roentgenograms, or possibly supplemented by sagittal tomography can be increased, whereby myelography will in many cases become superfluous

ELA THOMAS M and MACLI G B Phlebography in the Klippel Trenaunay syndrome Acta radiol Diagnosis 15 (1974), 43

The phlebographic findings in 14 patients with Klippel Trenaunay syndrome are described The most important finding is absence or hypoplasia of the deep venous system Other findings include extensive varicose veins dilated venous trunks and enlarged perforating veins Surgical interruption of superficial veins in the presence of an abnormal deep system may worsen the symptoms It is suggested that the term Klippel Trenaunay syndrome should be retained but employed exclusively for patients with the triad of unilateral varicose veins cutaneous naevi and hypertrophy of bone and soft tissues without arterio venous fistula

WATANABE K, KAWAHIRA K and MATSUURA K Scintigraphy as a screening test for carcinoma of the pancreas Acta radiol Diagnosis 15 (1974), 57

An investigation was performed in 196 cases to evaluate scintigraphy as a screening test in the diagnosis of carcinoma of the pancreas Ninetytwo per cent of 39 cases of malignancy had a localized or total defect in the scintigram although the false positive rate for the material was 43 per cent On the other hand normal scintigrams excluded carcinoma The test appears to constitute a useful means for the diagnosis in this condition

BOIJSEN E KAUDE J and TYLÉN U Radiologic diagnosis of ileal carcinoid tumours Acta radiol Diagnosis 15 (1974) 65

In a material of 15 patients with ileal carcinoid tumours calcifications were demonstrated in 7 cases Angiography in 14 of the cases revealed narrowing or occlusion of the superior mesenteric artery The angiographic diagnosis cannot be made until the ileal carcinoid has extended to the mesentery and infiltrated the lymph nodes Metastases to the liver are always richly vascularized and were present in 7 of 9 patients

MATTAMO J I and CARLSSON E Organic tricuspid valvular insufficiency in children Acta radiol Diagnosis 15 (1974) 83

Two cases of organic tricuspid valve insufficiency in children are reported The significance of quantitation is stressed and a cineradiographic method for measurement of the regurgitant flow applied in one of the cases is described and discussed

DEIGHGRÄBER I REICHMANN S and BUREN M Film quality in mammary radiography *Acta radiol Diagnosis 15 (1974) 93*

A comparison has been made between a series of two roentgenograms of the female breast one intended for 90 second processing the other, containing more silver for 6 minute processing in a roll machine The latter produced considerably better detail The method as to how industrial film with a high silver content may be incorporated into the processing routine of the average clinic is discussed

MILMAN N and STAGE P High dose urography in advanced renal failure II Influence on renal hepatic function Acta radiol Diagnosis 15 (1974) 101

Renal and hepatic function was evaluated in the periods before and after high dose urography with an average of 2 5 ml/kg Urografin 76 in 47 patients in end stage uremia by frequent recording of serum creatinine endogenous creatinine clearance and serum glutamic pyruvic transaminase A significant transient decrease in renal function in one patient indicating temporary dialysis was noted No change in hepatic function was observed

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SARCOMAS OF THE NASOPHARYNX

A DE SCHRYVER

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From Radiumhemmet (Director Prof J Einhorn) and the Department of Tumour Pathology (Director Prof G Moberger), Karolinska Sjukhuset, 104 01 Stockholm Sweden

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The observations presented constitute a clinical investigation of a comparatively large series of cases of sarcomas of the nasopharynx, followed up systematically for 5 to 32 years The histology was re-evaluated and its significance in the prognosis of the condition considered in rather more detail in reticulum cell sarcomas

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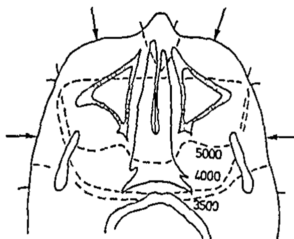


Fig. 1. Example of computed dose distribution at nasopharynx level.

Material. About 660 cases of malignant nasopharyngeal tumours were accepted for treatment during the period 1937 to 1964. A careful review of the histology left 140 cases of sarcoma for investigation. Another 14 cases had been classified as sarcomas but were excluded due to the poor technical quality of the slides.

Treatment. This in all cases but 3, that received chemotherapy, consisted primarily in roentgen therapy. The technique was basically constant and has been described in detail by DE SCHRYVER et coll. (1971).

The entire course covered 20 to 40 days, the estimated tumour dose ranging roughly from 5 000 to 6 000 rad (Fig. 1). If the changes were considered as persisting 6 to 8 weeks after completion of the course, a 50 mg radium source filtered by 4 mm Al, was applied locally for between 3 and 8 hours with approximately 1 000 to 2 500 rad at 0.5 cm. If treatment had to be given to both sides of the nasopharyngeal cavity, the applications were made on two consecutive days.

It was not customary during the period covered by this investigation to give treatment to the neck if no clinical involvement of the cervical nodes were evident. The lateral retropharyngeal or Rouvière's nodes were of course always included in the target volume together with the primary lesion.

Palpable nodes indicated that the side involved of the neck had to be included in the treatment. These cervical fields were in fact a continuation of the lateral nasopharyngeal field extending to the clavicle (Fig. 2). The skin doses varied from 1 500 to 3 500 R over 20 to 40 days. Recurrences or residual nodes could afterwards still receive further treatment either with more conventional roentgen irradiation or with teleradium (after 1957 with telecobalt) depending upon the condition of the skin after the previous dose.

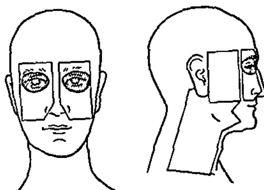


Fig 2 The treatment fields The cervical field, where used formed a downward prolongation of the lateral nasopharyngeal portal

Method The patients were re-examined at regular, more or less frequent intervals after treatment but never less than at least once a year, none was lost to control All were available for 5 year survival assessment, the times being calculated from the first day of treatment Survival rates were corrected for intercurrent deaths only Patients were considered to be clinically cured, or to have died of intercurrent disease only if they had been clinically and radiologically free from malignancy for at least one year (for the first year this was calculated from the first day of treatment) and autopsy, if any, failed to reveal any evidence of recurrence If the clinical condition leading to death were unknown, the patient was considered to have died from the sarcoma even if he had been symptom free for more than a year

All available slides (haematoxylin eosin) were reviewed blindly by the author together with a pathologist without previous knowledge of the clinical course of the disease The diagnosis was also checked by another two pathologists if any doubt existed

It was found that all the 140 cases available for assessment could be classified into one of the following 4 categories (1) Sarcomas of embryonal, vascular or connective tissue origin (8 cases), (2) plasmocytosarcomas (13 cases), (3) lymphocytic and lymphoblastic lymphomas (lymphosarcomas) (5 cases) and (4) reticulum cell sarcomas (114 cases)

Although in recent years the term reticulum cell sarcoma has become a somewhat controversial one (GALL & RAPPAPORT 1958, LUKES 1967), mainly among American pathologists, it is still widely used to describe a form of malignant lymphoma in which the proliferating tissue is composed mainly 'of either neoplastic primitive reticular cells or their histiocytic derivatives' (RAPPAPORT 1964 b) The presence of argentophilic ('reticulin') fibres is not required for the diagnosis In poorly differentiated cases, the growth may be difficult to

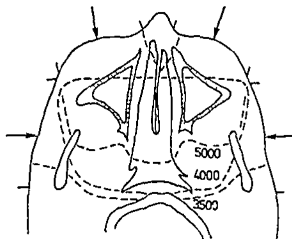


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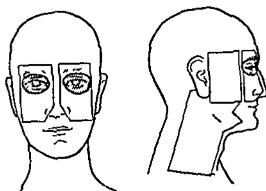


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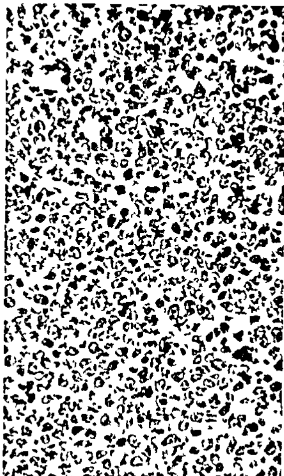


Fig. 3

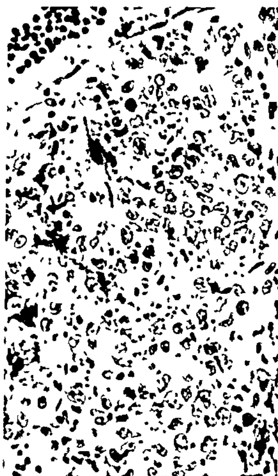


Fig. 4

Fig. 3. Reticulum cell sarcoma, stem cell lymphoma type. Monomorphous large tumour cells. Haematoxylin eosin staining. Photomicrograph ×350.

Fig. 4. Reticulum cell sarcoma, histiocytic lymphoma type. Tumour cells of varying size, polymorphous cellular structure. Haematoxylin eosin staining. Photomicrograph ×350.

distinguish from anaplastic carcinoma which, however, has a tendency to infiltrate more in separate strands than the sarcoma. The diagnosis had sometimes to be made rather by exclusion of other possibilities such as a poorly differentiated carcinoma or lymphoblastic lymphoma.

The validity of the concept of 'reticulum cell sarcoma', introduced by ROULET in 1930 ('Retothelsarkom') was challenged by GALL & MALLORY (1942) on the grounds that few authors, if any, seemed to agree as to the true nature or even the morphologic characteristics of the involved cell. According to GALL (1958) the reticulum cell 'appears to be a myth, and the retention of the term

would seem to serve no useful purpose' He recommended the tumours commonly called reticulum cell sarcoma should be termed by the type of cell from which, in all probability, they are derived the primitive mesenchyme cell ('lymphoma, stem cell type') or the histiocyte ('lymphoma, histiocytic type')

The very concept of reticulum cell sarcoma may still be widely accepted as a meaningful entity, nevertheless the fact that at least two different subtypes may be distinguished, is becoming increasingly recognised These, according to GALL & MALLORY (1942), RAPPAPORT (1964 b) and LUKES (1967), should replace the classical 'reticulum cell sarcoma' and may be described, largely after RAPPAPORT (1964 a), as follows

Stem cell lymphoma (Fig 3) Malignant neoplasm of reticular tissue composed of primitive cells without appreciable histiocytic or lymphocytic differentiation The cells are large (15 to 35 μ), with varying amounts of, frequently pale staining, cytoplasm and large, round to oval, distinctly delineated, vesicular nuclei A small but discrete nucleolus is frequent Cell borders are often not evident, reticulum fibrils are comparatively sparse Some degree of histiocytic differentiation may sometimes render an attempt of classification difficult

Histiocytic lymphoma (Fig 4) Malignant neoplasm of reticular tissue composed predominantly of neoplastic histiocytes which possess the power of phagocytosis, fibril formation or both Again, the cells are larger than lymphocytes, but in the description of GALL & MALLORY (1942), slightly smaller than stem cells, in fact, they may vary widely in size (LUKES 1967) and shape, depending upon their degree of differentiation The distinct nuclear membrane is often thicker than that of the stem cells, it may have chromatin condensations on its inner surface The nucleoli are often considerably larger than in stem cells In more differentiated cases the cellular outlines may become irregular and abundant argentophilic fibril formation may eventually be observed The histologic diagnosis of types of sarcomas other than reticulum cell sarcomas offered no difficulties From a differential diagnostic point of view, lymphoblastic lymphomas may be distinguished from stem cell lymphomas by the constant presence of mature lymphocytes and less frequent nucleoli in the former tumour type

Staging It would appear that most of these neoplasms behave rather like epithelial tumours in their mode of spread, any nodes below the clavicle were considered as metastases and, in fact all the 'localized cases could be classed as in stages I and II according to PETERS (1963), where stage I corresponds to the absence of cervical gland involvement and stage II to the presence of cervical

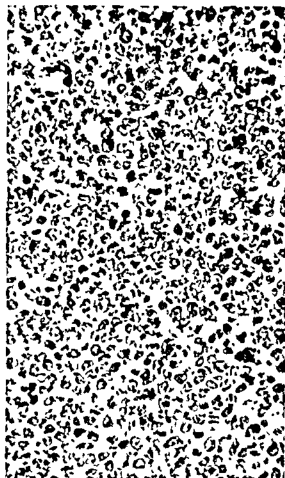


Fig 3

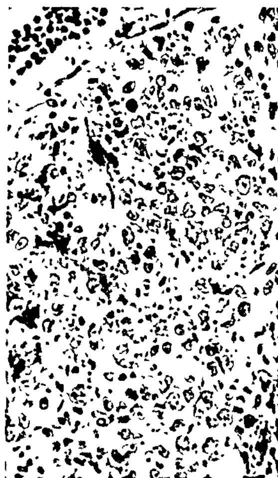


Fig 4

Fig 3 Reticulum cell sarcoma, stem cell lymphoma type Monomorphous large tumour cells Haematoxylin-eosin staining Photomicrograph ×350

Fig 4 Reticulum cell sarcoma, histiocytic lymphoma type Tumour cells of varying size polymorphous cellular structure Haematoxylin-eosin staining Photomicrograph ×350

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Table 1

Reticulum cell sarcomas Survival according to clinical stage

	No of cases	Dead from interc disease	Left for assessm	5-year survival	
				No	%
Cases without palp nodes (I)	15	1	14	8	57
Cases with unilateral nodes	28	2	26	12	46
Cases with bilateral nodes	59	0	59	16	27
All localized cases (I, II)	102	3	99	36	36
All generalized cases (III)	12	0	12	0	0
All cases (I II III)	114	3	111	36	32

involvement of the cervical nodes when they were first seen One patient survived for 27 years although with a spread to the axillary and mediastinal nodes when she was admitted for treatment The other 4 patients died from 0 to 2 years after treatment, one in lymphatic leukaemia, the others in generalized lymphoma

Reticulum cell sarcomas One hundred and fourteen patients were given this diagnosis, among them 69 males (= 60 5 per cent) and 45 females (= 39 5 per cent), their ages ranging from 8 to 79 years, the age distribution appears in Fig 5 a The over all 5-year survival was 32 per cent (Table 1) With 2 exceptions all these patients were accepted for primary radiation therapy to the nasopharynx, although in 10 of them the condition was known to have produced some form of distant metastases When allowance is made for 3 intercurrent deaths, the survival for the cases without known distant metastases becomes 36 per cent For the patients without any node involvement (stage I) the 5 year survival becomes 8 out of 14 (57 per cent) With bilateral node involvement, the survival drops to 16 out of 59 (27 per cent)

Sixty three cases could be classified histologically as stem cell type lymphoma, 46 as histiocytic type lymphoma In 5 cases classification proved to be impossible The prognosis proved to be worse for the former as compared to the latter Five year survivals were only 29 per cent for the stem cell as against 50 per cent for the histiocytic type (stages I + II) (Table 2)

A diagnosis of a second growth was made either previous to or after the treatment of the nasopharyngeal lesion in 12 patients (other reticulum cell sarcoma localizations were not considered new tumours) (Table 3)

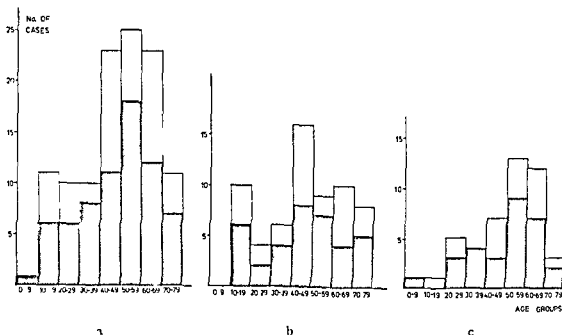


Fig 5 Reticulum cell sarcoma, age and sex distribution a) All cases, b) stem cell type c) histiocytic type ☐ males ☐ females

nodal disease, with the 'generalized' cases in stage III However, the staging was performed a posteriori and it should be borne in mind that for most patients only routine clinical examinations, at best supplemented with roentgen films, were available A number of cases were presumably understaged

Results

Sarcomas of embryonal, vascular and connective tissue origin Of the 8 patients, 2 were females and 6 males, their ages ranging from 3 to 67 years with 5 under the age of 15 Out of these 8 cases, only 2 survived for over two years, one of them for 8 years Of the remaining 6, 1 lived for a year and the other 5 patients under a year

Plasmocytosarcomas The diagnosis of a solitary, nasopharyngeal plasma cell tumour was made in these 13 patients Ten were males and 3 females, their ages ranging from 48 to 78 years Five had regional lymph node metastases, 5 survived for 5 years or more

Lymphocytic and lymphoblastic lymphomas Only 5 patients comprised this group, 4 males and 1 female, their ages ranging from 12 to 73 years All had

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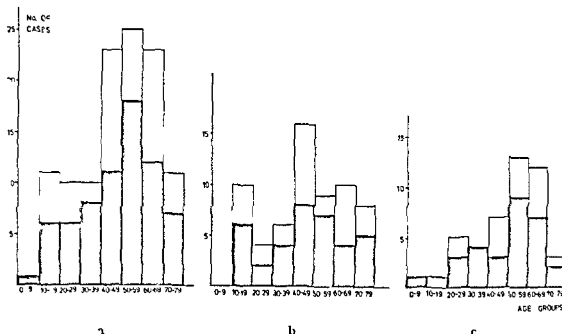


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Lymphocytic and lymphoblastic lymphomas Only 5 patients comprised this group, 4 males and 1 female, their ages ranging from 12 to 73 years All had

Table 3

Reticulum cell sarcoma Second primary tumours

Stem cell type			Histiocytic type		
Case	Sex	Second tumour	Case	Sex	Second tumour
19	F	Carcinoma uterine cervix	225	M	Carcinoma, colon
75	F	Carcinoma, uterine cervix	261	M	Carcinoma colon
107	F	Carcinoma uterine cervix	93	M	Carcinoma, rectum
87	M	Carcinoma lip	51	M	Carcinoma, tongue
209	M	Neurofibrosarcoma of parotid	56	F	Liposarcoma
			77	M	Gastric carcinoma*
			206	F	Gastric carcinoma*

* Biopsy or autopsy report not available

A typical example was a man, aged 58, who had complete regression of the tumour on treatment, but developed a recurrence one year later He refused treatment but remained nevertheless well until he died, 5 years later, in an accident

Most of these tumours were radiation sensitive and responded well to treatment, but the author would agree with ENNUYER et coll (1963) that occasionally regression may be slow and require high doses In one patient it took almost half a year after the termination of radiation therapy for the lesion in the nasopharynx to disappear completely

Five of the 13 patients presented on admission unilateral or bilateral involvement of the cervical lymph nodes so that the writer cannot wholly agree with TODD (1965) that the regional lymph nodes need not be included within the treated volume as a routine In one of the 8 patients without clinical involvement of the cervical lymph nodes on admission, regional metastases developed after completion of the treatment of the nasopharynx To include at least the upper part of the neck would seem a relatively simple prophylactic measure with modern high voltage equipment

None of these patients had received chemotherapy, some not at all, others with drugs or in doses that would be considered inadequate today, it would seem highly probable that more or less prolonged remissions could have been achieved if satisfactory chemotherapy had been administered

The possibility that the cases which eventually developed bone involvement were, in fact, cases of primary multiple myeloma (with a much poorer prognosis) cannot be entirely excluded

Table 2

Reticulum cell sarcomas, stages I and II. Survival according to cytologic type

	No. of cases	Mean age (yrs)	No. of cases with general disease (III)	No. dead of interc. dis.	Left for assessm.	5-year survival	
						No.	%
Stem cell type	63	50	8	0	55	16	29
Histiocytic type	46	46	3	3	40	20	50
Reticulum cell sarcomas (all cases)	114	48	12	3	99	36	36

Discussion

Embryonal, vascular and connective tissue sarcomas. These tumours, appearing mainly in children and young adults, are rare and apparently highly malignant. All of the 8 patients died, one of them (a 54-year-old woman with angio sarcoma) surviving for 8 years free from local disease but dying eventually from distant metastases. The remaining 7 patients all died from the local condition. Five patients received treatment up to the skin tolerance limits, the primary growth was slow in disappearing and recurred locally in 3 of them. The doses in the remaining 3 patients were only palliative in the first place, due either to metastases or to advanced local disease. It would not seem unreasonable to believe that some of these patients might have done better with modern high-voltage equipment and homogeneously distributed tumour doses up to the limit of normal tissue tolerance.

Plasmocytosarcomas. In this series, the youngest patient with a plasma cell tumour was 48 years and the oldest 78, making this type of malignancy a disease of the older age groups. This is consistent with the general consensus of the literature, as is the male predominance (in the present series: 10 males and 3 females). The 5-year survival rate is about 40 per cent (5/13), with 2 of the patients living for over 20 years. Three of the 8 patients who died within 5 years, had bone involvement which was discovered 6, 10 and 22 months after initiation of the primary treatment. One of the 5-year survivors developed massive invasion of the base of the skull. Three others died from intercurrent disease. Even among the patients who cannot be considered as having been cured, 5 died of causes that were obviously not related to the malignancy; this was perhaps not surprising in view of their age (58, 63, 71, 76 and 78 years, respectively).

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87	M	Carcinoma lip	51	M	Carcinoma tongue
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			77	M	Gastric carcinoma*
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Table 4

Reticulum cell sarcoma Difference in clinical behaviour between histologic subgroups

	Stem cell type	Histiocytic type
No of cases subjected to radium application	28 63 (44 %)	10/46 (22 %)
No of cases with recurrent nodes after primary treatment to the neck	12/53 (23 %)	6/39 (15 %)
Average length of interval between primary treatment and distant spread	9 months	26 months
No of cases where appearance of metastases was delayed 1 year	6/29 (21 %)	8/20 (40 %)

tempted to see a biphasic age distribution, but is would appear that the number of cases was too small to permit of any conclusions No marked differences were evident either in the frequency or pattern of the metastases, nodal or distant

A second malignancy developed either before or after the reticulum cell sarcoma in 12 cases (11 per cent) (7 out of 46 in the histiocytic and 5 out of 63 in the stem cell group, Table 3) This would be rather more than the figure of 2 per cent (7 out of 368 cases) given by MOERTEL (1966) and probably would have to be explained by a difference in observation time In 2 cases of carcinoma of the stomach, however, neither biopsy nor autopsy was reported

The 5-year survival in the reticulum cell sarcoma group as a whole was 32 per cent (Table 1) Both subgroups present, however, a marked difference, statistically significant at the 5 per cent level (5 % > p > 1 %) 29 per cent for the stem cell lymphomas against 50 per cent for the histiocytic lymphomas, if only stages I + II are considered (Table 2), and 25 per cent against 47 per cent if stage III cases are included (Fig 6) Cure rates calculated as expressions of symtom free survival were as expected, slightly lower for both groups during the first few years although the curves for survival and cure rates became identical after year 4 Cure rate differences were not significant (A recurrence or metastases later developed in 4 patients considered cured by the criteria mentioned earlier Two had stem cell and 2 histiocytic type lymphoma The recurrence appeared in 3 patients during the second year after the primary treatment, in the fourth patient (histiocytic type) during the 6th year All 4 patients lived for more than ten years after treatment of the further lesion and remained symptom free) These differences in survival and cure rates were apparently not due to a higher proportion among the histiocytic group of stage I lesions, which was about 11 per cent (5 out of 46) for the histiocytic, and 13

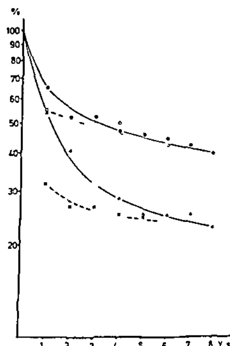


Fig 6 Reticulum cell sarcoma survival curves ●——● survival rate, histiocytic type O - O cure rate, histocytic type △——△ survival rate stem cell type × - × cure rate stem cell type

Lymphocytic and lymphoblastic lymphomas Although these tumours are radiation sensitive, all of the 5 cases did badly Of course, radiation therapy would now be supplemented by active chemotherapy in the later stages, presumably with better results However, this group is too small to allow of any conclusions

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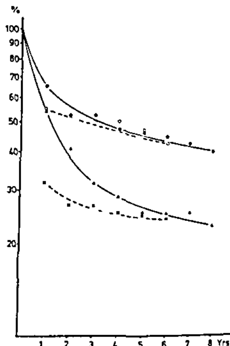


Fig 6 Reticulum cell sarcoma, survival curves ●——● survival rate, histiocytic type O- - -O cure rate, histiocytic type △——△ survival rate, stem cell type X- - -X cure rate, stem cell type

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Table 6

Reticulum cell sarcoma Subsequent development of cervical nodes in untreated neck

	No of patients	Per cent
Stem cell type	5/23	–
Histiocytic type	3/22	–
All patients	9 49	18

developed distant spread, 40 per cent (8 out of 20) were detected after a delay of 1 year, whereas only 21 per cent (6 out of 29) were delayed for a year in the stem cell group

It would appear from these observations that the lesion described as the histiocytic type of reticulum cell sarcoma carries a better prognosis than the stem cell type, not only because of the later appearance of distant spread but probably also because of its greater response to irradiation Another way of illustrating this is to compare the fate of all stage I cases all treatment failures (5 out of 8) without exception belong to the stem cell group, whereas no case of a localized histiocytic lymphoma was lost

Patients between 15 and 40 years of age had a better prognosis 14 out of 27 (52 per cent) survived for 5 years or more, against only 22 out of 79 (28 per cent) of patients over 40 (Table 5) Apparently this is due mainly to the stem cell cases where the difference is as marked as 9 out of 16 (56 per cent) for patients under, against 7 of 43 (16 per cent) of patients over 40 years This partly confirms the findings of FULLER (1967) and of PETERS (1963) whose patients between 20 and 39 did best In this last series patients under 20 did much worse as a group Among our own patients, none of the 5 under the age of 15 lived for more than 1 year

At the time when most of these patients were treated, it was not customary in this institute to make individual treatment plans for roentgen therapy As it was obviously impossible to reconstruct the distribution of the absorbed radiation doses for more than a few of those still living, it appeared somewhat hazardous to speculate about the tumour doses to the nasopharynx and their significance for the survival figures in the material The doses applied were often obviously adequate to control the primary tumour, in part possibly due to the local radium applications The primary treatment of the cervical regions with doses of 3 000 to 3 500 R/40 days at the very most (often much less), dictated by the tolerance of the skin, is considered however by most authors to be inadequate This is illustrated in the present series by a rate of 15 per cent (histiocytic type) and 23

Table 5

Reticulum cell sarcoma Five year survival according to age (patients dead with intercurrent disease, without evidence of tumour, not included)

	Stem cell type (63)	Histocytic type (43)	All patients (111)
Patients under 15 years	0/4	0/1	0/5
Patients from 15 to 40 years	9/16 (56 %)	5/10 (50 %)	14/27 (52 %)
Patients over 40 years	7/43 (16 %)	15/32 (47 %)	22/79 (28 %)

per cent (8 out of 63) for the stem cell cases, nor to a difference in age distribution (mean age for the histiocytic cases 46 2 against 49 8 years for the stem cell cases) It would, on the other hand, not be wholly unexpected if it were assumed that the stem cell type of lymphoma is the more undifferentiated but this alone hardly constitutes an explanation Basically, better survival might be expected from radiation therapy if (1) the lesion were more radiation sensitive or (2) produced metastases at a later stage or (3) both conditions were present

It would seem extremely hazardous, if not impossible, to make any a posteriori estimation of the sensitivity of these cases from the clinical data in their files It is known, however, that 6 to 8 weeks after completion of the external radiation therapy, the nasopharynx was routinely re-examined, residual or possible maligancy indicated the application of radium It would thus seem that the frequency of radium applications could be used as an—admittedly coarse—indicator of the sensitivity of the lesion to the applied external treatment Twice as many patients received radium applications in the stem cell group as in the histiocytic group (Table 4) This distribution would appear strongly in favour of a greater responsiveness of the histiocytic type of lymphoma to primary radiation treatment in comparison to the stem cell type The fact that the stem cell cases carried a somewhat greater tendency to lymph node recurrence after radiation treatment (Table 4), a difference not statistically significant, would be in accordance with this assumption

Comparison of the interval between the primary treatment and the diagnosis of distant metastases revealed an average figure of 9 months for the stem cell type and 26 months for the histiocytic type Quite independently from the tumour response to the treatment, this ought to contribute to better survival rates To exclude the possibility that these figures could be due mainly to one or two long survivors, the numbers of patients developing metastases after a delay of at least 1 year were compared Again the figures were clearly balanced in favour of the histiocytic lymphoma (Table 4) of all the cases that eventually

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per cent (stem cell type) local recurrences (Table 4). In agreement with NEWALL & FRIEDMAN (1970), PETERS (1963), WANG (1969) and FULLER (1967), it would appear that at least 5 000 rad should be the aim whenever there are palpable nodes. Moreover, nothing seems to be gained by not treating both cervical regions as a matter of routine up to at least 4 000 rad even if they are clinically free from disease; this can now easily be achieved with megavoltage equipment. Unlike FULLER (1967), PETERS (1963) and HOPF-STONE (1969) feel that no advantage is to be expected from 'prophylactic' irradiation of uninvolved node regions. As none of the present patients was treated in this manner, this series cannot in any way contribute to the argument. Lymph node metastases however eventually developed in untreated necks in 9 out of 49 patients (18 per cent) (Table 6). Even if it is admittedly dubious whether primary treatment of the entire neck in adequate dosage would have had any effect on the actual survival figures, it would almost certainly have prevented at least some of the secondary localizations and the resulting renewed course of treatment and hospital attendance. As clinical palpation is inadequate to exclude the presence of metastases primary treatment of the neck should be recommended.

In the GALL & MALLORY monograph (1942), covering all possible localizations, the 5-year survival rates mentioned for stem cell and histiocytic types were 14 per cent (out of 56 cases) and 11 per cent (out of 71 cases) respectively. It is obvious, however, from reading their paper that the treatments applied (600 R) must usually have been grossly inadequate and makes any comparison with the treatment results in other series difficult. Although the doses applied were higher, the same may hold true for the cases reported by HILTON & SUTTON (1962), where the survival rate for both types was 20 per cent (stem cell) and 15 per cent (histiocytic), respectively. The present investigation was concerned only with sarcomas originating in or involving the nasopharynx. As NEWALL & FRIEDMAN (1970) indicated, reticulum cell sarcomas may present with different biologic characteristics (for example radiation sensitivity) according to the site of origin. The present results may therefore not necessarily apply to other localizations of the disease, nor are they necessarily in contradiction with the observations of MATHE et coll. (1970) who, in their report on 110 cases of 'reticulosarcomas' were unable to discover any difference in 5 year survival between their 'histioblastic' and 'histiocytic' subtypes. Both the survival rates (10 and 12.5 per cent, respectively) and the fact that 17.5 per cent of their 'histioblastic' cases eventually became transformed into acute monoblastic leukaemia (a transformation never evident in the present material), clearly indicate that the conditions at their investigation were altogether different and that basic discrepancies must exist in the pattern known as reticulum cell sarcoma.

The question whether any closer relationship exists between the different types of (nasopharyngeal) lymphomas, for example whether the disease can change from one type into another in the same patient in the course of its evolution, or whether two or more types can coexist simultaneously, has not been broached As a rule, only one or two biopsies were available for each case which would have precluded any attempt to perform pathologic investigations of the serial type

Acknowledgements

The author takes this opportunity of expressing his indebtedness to the late Prof L. Santesson who so kindly reviewed the slides and of thanking Prof G Moberger and Dr G Soderberg for their help

SUMMARY

Treatment results were assessed in a series of 140 consecutive cases of non epithelial tumours of the nasopharynx the most important group (114 cases) of which consisted of so called reticulum cell sarcomas Two histologic sub groups were distinguished, the stem cell and histiocytic types The latter type was the less malignant the 5 year survival being 47 against 25 per cent for the stem cell type although for which the prognosis was better between the ages of 15 and 40

ZUSAMMENFASSUNG

Die Behandlungsergebnisse einer Serie von 140 aufeinanderfolgenden Fallen nicht-epithelialer Tumoren des Nasopharynx bei denen die bedeutungsvollste Gruppe (114 Falle) aus sogenannten Retikelzellsarkomen bestand wurden festgestellt Zwischen zwei histologischen Untergruppen wurde unterschieden einem Stammzelltyp und einem histiozytaren Typ Letzterer war weniger malign die 5 Jahres Überlebensrate betrug 47 % gegenuber 25 % bei dem Stammzelltyp obwohl fur diesen die Prognose zwischen Altern von 15 und 40 Jahren gunstiger war

RÉSUMÉ

L auteur a evalue les resultats therapeutiques sur une serie de 140 cas consecutifs de tumeurs non-epitheliales du nasopharynx dont le groupe le plus important (114 cas) etait celui des sarcomes dits à cellules reticulaires Il a distingue deux sous groupes histologiques, les types a cellules souches et histiocytaires Ce dernier type est le moins malin avec une survie à 5 ans de 47 pour cent contre 25 pour cent pour le type à cellules souches bien que le pronostic de celui ci soit meilleur entre 15 et 40 ans

REFERENCES

BERDAL P and POPPE E Malignant tumours of the nasopharynx Preliminary results of treatment with high energy roentgen rays (31 MeV betatron) Acta radiol 58 (1962), 241

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In the GALL & MALLORY *monograph (1942), covering all possible localizations*, the 5-year survival rates mentioned for stem cell and histiocytic types were 14 per cent (out of 56 cases) and 11 per cent (out of 71 cases) respectively It is obvious, however, from reading their paper that the treatments applied (600 R) must usually have been grossly inadequate and makes any comparison with the treatment results in other series difficult Although the doses applied were higher, the same may hold true for the cases reported by HILTON & SUTTON (1962), where the survival rate for both types was 20 per cent (st m cell) and 15 per cent (histiocytic), respectively The present investigati
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The question whether any closer relationship exists between the different types of (nasopharyngeal) lymphomas, for example whether the disease can change from one type into another in the same patient in the course of its evolution, or whether two or more types can coexist simultaneously, has not been broached. As a rule, only one or two biopsies were available for each case which would have precluded any attempt to perform pathologic investigations of the serial type.

Acknowledgements

The author takes this opportunity of expressing his indebtedness to the late Prof. L. Santesson who so kindly reviewed the slides and of thanking Prof. G. Moberger and Dr. G. Soderberg for their help.

SUMMARY

Treatment results were assessed in a series of 140 consecutive cases of non epithelial tumours of the nasopharynx the most important group (114 cases) of which consisted of so called reticulum cell sarcomas. Two histologic sub groups were distinguished, the stem cell and histiocytic types. The latter type was the less malignant, the 5 year survival being 47 against 25 per cent for the stem cell type, although for which the prognosis was better between the ages of 15 and 40.

ZUSAMMENFASSUNG

Die Behandlungsergebn[illegible]
lialer Tumoren des [illegible]
sogenannten Retikel[illegible]
Untergruppen wurd[illegible]
Letzterer war weniger [illegible] die 5 Jahres Überlebensrate betrug 47 % gegenuber 25 % bei dem Stammzelltyp, obwohl fur diesen die Prognose zwischen Altern von 15 und 40 Jahren gunstiger war.

RÉSUMÉ

L'auteur a evalue les resultats therapeutiques sur une serie de 140 cas consecutifs de tumeurs non epitheliales du nasopharynx dont le groupe le plus important (114 [illegible]
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REFERENCES

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FROM THE DEPARTMENTS OF OTOLARYNGOLOGY AND TUMOUR PATHOLOGY AND RADIUMHEMMET, KAROLINSKA SJUKHUSET, AND THE DEPARTMENTS OF CLINICAL RADIATION PHYSICS, NATIONAL INSTITUTE OF RADIATION PROTECTION, 104 01 STOCKHOLM, AND PATHOLOGY, SODERSJUKHUSET, 100 64 STOCKHOLM, SWEDEN

POORLY DIFFERENTIATED SOLID PAROTID CARCINOMA

C Blanck, A Backstrom, C-M Eneroth and P Å Jakobsson

A histologic re-examination and reclassification of a series of 1 678 parotid tumours revealed that poorly differentiated solid carcinomas constituted the largest group of malignant neoplasms with 75 cases (4 5 per cent) Areas with histologic structures characteristic of poorly differentiated solid carcinoma may occur in other types of malignant parotid growths such as carcinoma in pleomorphic adenoma, adenoid cystic carcinoma and high grade malignant adenocarcinoma The structures characterizing these better defined types may, however, be overlooked if removal of the tumour is so incomplete that only a part of it is available for examination The relatively high incidence in the present series of poorly differentiated solid carcinomas may thus be explained by the fact that the surgical procedure was usually only of a minor nature

Modern classifications define the different types of salivary gland tumours on the differentiation of the histologic structures (Blanck et coll 1967, 1971, *Eneroth et coll 1966, 1968, Jakobsson et coll 1968*)

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ENNUYER A, BATAINI P, CHAVANNE G et HÉLARY J: Les plasmocytomes des voies aéro digestives supérieures. Ann Radiol 6 (1963) 741.

FULLER L M: Results of intensive regional radiation therapy in the treatment of Hodkin's disease and the malignant lymphomas of the head and neck. Amer J Roentgenol 99 (1967) 340.

GALL E A: The cytological identity and interrelation of mesenchymal cells of lymphoid tissue. Ann N Y Acad Sci 73 (1958) 120.

— and MALLORY T B: Malignant lymphoma. A clinicopathological survey of 618 cases. Amer J Path 18 (1942) 381.

— and RAPPAPORT H: Seminar on diseases of lymph nodes and spleen. *In* Proc of 23rd Seminar Amer Soc clin Pathol, p 85. Edited by J R McDonald 1958.

HILTON G and SUTTON P M: Malignant lymphomas: classification, prognosis and treatment. Lancet 1 (1962) 283.

HOPE STONE H F: The treatment of the reticuloses. Brit J Radiol 42 (1969) 770.

LUKES R J: A review of the American concept of malignant lymphoma. The evolution of a modern classification. *In* Progress in Lymphology. Proceedings of the International Symposium on Lymphology, Zurich, Switzerland, July 19—23, 1966, p 109. Edited by A Ruttimann. Georg Thieme, Stuttgart 1967.

MATHE G, GERARD MARCHANT R, TEXIER J L, SCHLUMBERGER J R, BERUMEN L and PAINTRAND M: The two varieties of lymphoid tissue "reticulosarcomas", histiocytic and histioblastic types. Brit J Cancer 24 (1970) 687.

MOERTEL CH G: Multiple primary malignant neoplasms. Their incidence and significance, p 49. Springer Verlag, Berlin, Heidelberg, New York 1966.

NEWALL J and FRIEDMAN M: Reticulum cell sarcoma. Part II. Radiation dosage for each type. Radiology 94 (1970) 643.

PETERS M V: The contribution of radiation therapy in the control of early lymphomas. Amer J Roentgenol 90 (1963) 956.

RAPPAPORT H (a): The histologic aspects of malignant lymphoreticular neoplasms. *In* The lymphoreticular tumours in Africa, p 174. Edited by F C Roulet. S Karger, Basel 1964.

— (b): Classification of neoplastic diseases of the reticular system. *In* The lymphoreticular tumours in Africa, p 394. Edited by F C Roulet. S Karger, Basel 1964.

ROULET F: Das primäre Retothelsarkom der Lymphknoten. Virchows Arch path Anat 277 (1930) 15.

SCANLON P W, RHODES R E, WOOLNER L B, DEVINE K D and McBEAN J B: Cancer of the nasopharynx: 142 patients treated in the 11-year period 1950—1960. Amer J Roentgenol 99 (1967) 313.

DE SCHRYVER A, WACHTMEISTER L and BÅRYD I: Ophthalmologic observations on long-term survivors after radiotherapy for nasopharyngeal tumours. Acta radiol Ther Phys Biol 10 (1971) 193.

TODD I D H: Treatment of solitary plasmocytoma. Clin Radiol 16 (1965) 395.

WANG C C: Malignant lymphoma of Waldeyer's ring. Radiology 92 (1969) 1335.

YEH S: A histological classification of carcinomas of the nasopharynx with a critical review as to the existence of lymphoepitheliomas. Cancer 15 (1962) 895.

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Submitted for publication 28 May 1973

carcinomas and other forms of poorly differentiated carcinomas to one tumour group whereas others consider certain of these variations as distinct histologic forms Furthermore, in earlier series there was often an overrepresentation of poorly differentiated solid carcinomas owing to the fact that minor operations were generally performed

The present work was aimed at the histologic and clinical features of poorly differentiated solid carcinomas in a series of cases with a long control period these features were compared with those of other types of parotid malignancy

Material

The investigation was based on an analysis of *1 678 histologically verified* tumours of the parotid gland treated from 1909 to 1958 The prerequisites for histologic re examination existed since slides or tissue blocks had been kept The *tissue blocks* had been *embedded in paraffin* and there were usually several blocks from each case After histologic re examination of the 1 678 neoplasms 299 were classified as malignant and 75 of them as poorly differentiated solid carcinomas These 75 tumours were investigated as to *local* recurrence, metastases, other clinical features and survival rate of the patients The follow up period was counted from the first histologic verification of the tumour The patients were followed by regular annual examinations twelve monthly reports from a physician or by annual communications from the patient, whenever necessary, the last mentioned were checked The survival rate is based on determinate groups which do not include patients lost to control or those dying without signs of the disease No patient in the present series was lost to control The grade of malignancy of the growth was calculated from the determinate survival rate which is thus based on the mortality in the disease The 75 patients in the series were operated on from 1922 to 1958 and all were traced up to at least 1964

Histology Most of the tumours were in accordance with the terms poorly differentiated and solid (Figs 1 to 5), in only 18 cases were there indications of differentiation in one direction or other These characteristics were however, not so prominent as to make it possible to place the growths in one of the distinctly defined tumour groups in the classifications presented by FOOTE & FRAZELL (1954) RAUCH (1959) and ENEROTH (1964)

Twelve of the 18 tumours with some suggestion of differentiation had indications of adenomatous structures (Fig 4) and 6 of these contained mucus The tissue involved was dark celled and similar to adenoid cystic carcinoma in 4

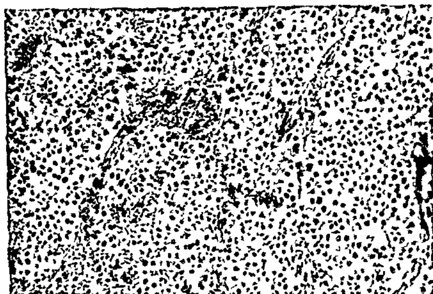


Fig 1 Poorly differentiated solid carcinoma Scanty stroma between large solid epithelial sheets H & E × 130

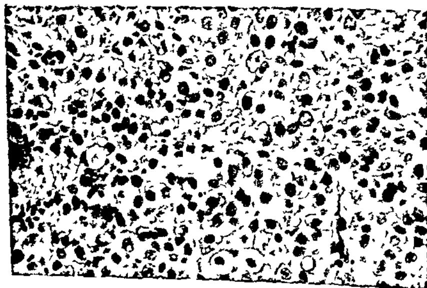


Fig 2 Same case At least five mitotic figures present in this field H & E × 325

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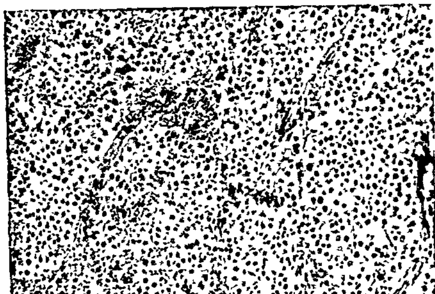


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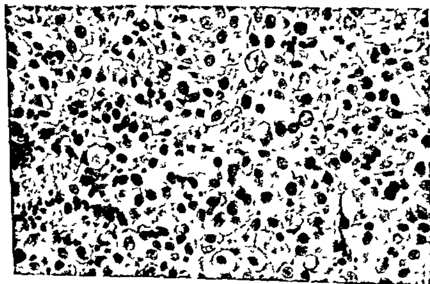


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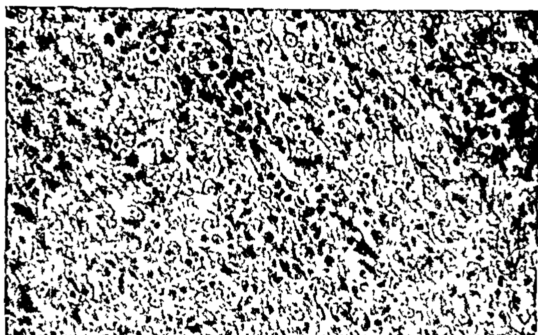


Fig 3 Poorly differentiated solid carcinoma Tumour cells growing in an almost syncytial fashion with lymphocytes in the stroma, similar to so-called lymphoepithelioma (Schmincke—Regaud) of the upper air passages H & E × 344



Fig 4 Poorly differentiated solid carcinoma The stroma more rich in collagen, possibly a few adenomatous indications in otherwise solid tumour sheets H & E × 145

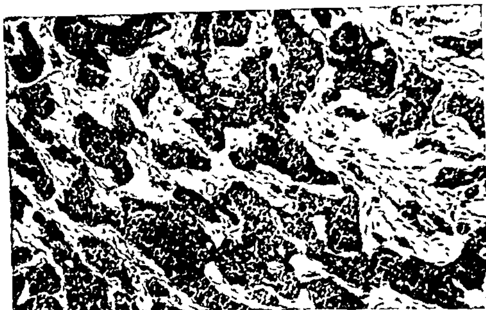


Fig 5 Poorly differentiated solid carcinoma Dark celled and small-celled tumour tissue similar to adenoid cystic carcinoma but solid H & E × 55

cases (Fig 5) although without adenomatous or cystic structures Some epidermoid differentiation was perhaps present in one case

The stroma was plentiful in 12 cases but not so luxuriant as to suggest a true fibromatous tumour component Peri- or intraneural malignancy was evident in 3 cases Vessels were involved in 3 cases and in one of them the vessel affected was of wide dimensions and thick-walled

Invasive malignancy was obvious in 44 cases and probable in a further 15 cases The tumour tissue available in 5 cases was too scanty for a decision to be made as to whether invasive growth existed or not In 11 cases, otherwise of the poorly differentiated carcinoma type, this could not be demonstrated (Table 1)

Abundant lymphoid tissue was present in 7 cases In at least one of them it was uncertain whether this was a lymphoid reaction in the malignant stroma or a metastatic nodule in a lymph node

Cytologic appearances The carcinoma cells varied in size and the cytoplasm was most often basophilic with the remainder eosinophilic The degree of polymorphism divided the tumours into three groups those with slight, moderate

Table 1

Poorly differentiated solid carcinoma of the parotid gland Histologic features and their influence on prognosis in 75 patients

Histologic features		No of patients	Died of	
			Tumour	Intercurrent disease
Infiltrative growth	Frank	44	31	8
	Probable	15	7	7
	Not demonstrated	11	7	1
	Inadequate specimen	5	3	2
Polymorphism	Slight	9	4	3
	Moderate	46	29	13
	Marked	10	7	1
	Inadequate specimen	10	—	—
Incidence of mitosis	High	27	16	9
	Low	48	32	9
Intravascular growth		3	3	0
Peri or intraneural growth present		3	3	0
Abundant lymphoid tissue reaction		7	3	3

and marked polymorphism (Table 1) Mitotic figures were more or less numerous in 27 cases, but few in 48 cases (Table 1) The presence of mucus in the tumour cells was tested with mucicarmine stain in 33 cases, and in 9 of these it was present

Clinical features Of the 75 patients with poorly differentiated solid carcinoma 34 were females and 41 males The age of the patients ranged from 17 to 84 years (mean 53 9) when the condition was detected, at the time of the first operation, when the tumour was first verified histologically, the range was 21 to 87 years (mean 57 6 years) The age yas about the same in men and women at the time of the first symptom (54 4 and 53 1 years respectively) and at the time of the first histologic verification of the tumour (56 6 and 58 7 years respectively) The mean interval between the initial symptom and the first histologic examination was 3 7 years

A history of pain was obtained in 20 patients (27 per cent) Persistent spontaneous paralysis of the facial nerve occurred in 18 patients (24 per cent) Ulceration of the skin or the external auditory meatus was evident in 9 patients (12 per cent) Symptoms and signs, apart from a palpable swelling in the parotid gland, were otherwise rare

Table 2

Poorly differentiated solid carcinoma of the parotid gland Clinical findings and their influence on prognosis in 75 patients

Clinical features		No of patients	Died of Tumour	Died of Intercurrent disease
Attachment	Complete	33	27	5
	Moderate	11	8	1
	Mobile	16	7	5
	Not stated	15	6	7
Consistency	Hard	30	26	3
	Firm	25	14	6
	Soft	2	0	2
	Not stated	18	8	7
Ulceration		9	7	2
Spontaneous facial nerve paralysis		18	18	0
Size (largest diameter)	< 3 cm	26	13	7
	3–5 cm	15	10	5
	> 5 cm	24	21	2
	Not stated	10	4	4
Pain	Present	20	16	2
	Absent	55	32	16

Table 3

Poorly differentiated solid carcinoma of the parotid gland with metastases in 43 out of 75 patients Influence on prognosis of metastases in different situations

Localization of metastases	No of patients	Died of Tumour	Died of Intercurrent disease
Regional lymph nodes alone	12	5	4
Distant alone	10	10	0
Both regional lymph nodes and distant	21	21	0
Regional lymph nodes total	33	26	4
Distant total	31	31	0

Table 4

Poorly differentiated solid carcinoma of the parotid gland Situation of distant metastases

Localization		No of patients
Lymph nodes	Infraclavicular	1
	Inguinal	2
	Axillary	3
Abdomen		6
Skeleton		11
Lungs		13
Pulmonary hilum		1
Mediastinum		2
Liver		6
Iliac fossa (left)		2
Mammary gland		1
Brain		4
Thyroid		1

The tumours were divided into three groups according to size at the first examination One group measured less than 3 cm in diameter (26 tumours), one between 3 and 5 cm (15 tumours) and one more than 5 cm (24 tumours) No data on size were recorded in 10 cases (Table 2)

The consistency of the growth was recorded in 57 cases and was described as hard in 30 cases, firm in 25 and soft in 2 cases No data on consistency were available in 18 cases (Table 2) Where data on attachment were recorded (60 cases), the tumour was described as completely attached in 33 cases, as moderately attached in 11 and as mobile in 16 cases (Table 2)

Local recurrence appeared in 26 of the 75 patients Eight of these 26 patients had more than one recurrence, in 3 patients two, in another 3 patients three, and in 2 patients four recurrences The time between the first verification of the growth by histology and the appearance of the recurrence was less than one year in 11 patients, between one and three years in 11, and between three and five years in 2 patients This interval was just over five years in one patient and in another could not be determined

Metastases were evident in 43 patients Twenty of them had metastases on admission and 23 patients developed metastases during the follow-up period Metastases to regional lymph nodes alone were registered in 12 patients and

distant metastases alone in 10, whereas both regional and distant metastases were present in 21 patients Thus regional lymph node metastases were present in a total of 33 patients and distant metastases in a total of 31 patients (Table 3) Distant metastases often appeared at several sites in the same patient, the lungs and the skeleton were the most common situations (Table 4) The skeletal metastases were distributed as follows with the number of patients in brackets ribs (6), skull (3), pelvic bones (3), mandible (1), femur (1), vertebrae (2), scapula (1), arm (1), hallux (1)

Treatment

Treatment of the poorly differentiated solid carcinoma of the parotid gland has consisted of a combination of surgery and radiation therapy The principles of therapy have, however, varied during the long period covered by the series

Surgery was performed in all 75 patients but in no less than 31 the procedure was of such a minor degree that it should be classed as excision biopsy The surgical intervention in 32 patients was denoted as extirpation and in 7 patients parotidectomy was performed In 3 of these 7 patients the parotidectomy was combined with cervical dissection The nature of the operation was not stated in 5 patients

Radiation therapy All patients received radiation therapy consisting of external irradiation, intracavitary radium therapy at operation, or a combination of the two

External irradiation was generally administered by short distance telegamma techniques using either teleradium units or ^{60}Co units with 6 to 7 cm between the radiation source and skin (WALSTAM 1965) Either one circular field 6 cm in diameter or a combination of two to six such fields, 6 to 7 cm apart were employed The estimated dose at 2 cm depth was generally of the order of 3 000 to 4 000 rad, given over 1 to 3 weeks Preoperative irradiation only was given to 15 patients, and postoperative irradiation only with short distance techniques to 30 patients The postoperative treatment was administered to one patient with a 15 MeV electron beam Preoperative treatment was given by conventional roentgen therapy in 2 cases and postoperative treatment to 7 patients Three patients received both pre- and postoperative external irradiation

Intracavitary irradiation was given by applying one to four 50 mg radium sources enclosed in a metal container, 10 mm in diameter, for 3 to 16 hours This type of therapy was applied in 17 patients, in 12 of them preoperative irradia-

tion, in 2 postoperative irradiation, and in 1 of them both pre- and postoperative irradiation were administered as well

Radiation sensitivity If a tumour that was easily palpable at the first examination was not definitely felt 6 weeks after ending irradiation, the sensitivity was considered to be high

In a small group of 11 primary tumours the tissue dose at different depths through the tissue could be calculated fairly accurately Preoperative irradiation was then given with short distance techniques over a circular field 6 cm in diameter The dose maximum at 2 mm depth was calculated to be 2 900 to 4 800 rad Treatment was fractionated over 3 to 5 days The dose at 1, 2 and 3 cm depth was 2 200 to 3 600, 1 700 to 2 800 and 1 200 to 2 100 rad, respectively In 5 of 11 patients the tumour disappeared clinically within 4 to 6 weeks of treatment At subsequent operation merely a small nonpalpable residue was present In the other 6 patients preoperative irradiation produced only moderate or no regression Not until the late 1950's was uniform treatment of poorly differentiated solid carcinoma of the parotid gland started as a routine with millionvolt radiation therapy and total parotidectomy combined with neck dissection

Prognosis

An attempt has been made to investigate the influence of different histologic features of the poorly differentiated solid carcinoma on the prognosis (Table 1) It became evident that the prognosis cannot be based on histologic structures such as infiltrative growth, polymorphism and mitotic figures It was however clear that all patients died of the disease when the growth had become intravascular and peri- or intraneural

The influence on the prognosis of different clinical features was also considered A large size of tumour and its attachment or hard consistency as well as dermal ulceration seem to imply a worsening of the prognosis (Table 2) A history of pain is also a bad prognostic sign, spontaneous facial nerve paralysis renders the prognosis most grave All 18 patients with spontaneous facial nerve paralysis died (Table 2) Local recurrence occurred in 26 patients, 19 (73 per cent) of whom died compared to 29 (59 per cent) of the 49 patients without local recurrence

The significance of the presence of metastases upon the prognosis is indicated in Table 3 Five of 12 patients with only regional lymph node metastases died compared with all 31 patients with distant metastases

The prognosis in poorly differentiated solid carcinoma was also investigated on the basis of the determinate survival rate during a follow-up period of 5 to

Table 5

Poorly differentiated solid carcinoma of the parotid gland Five to 25-year follow up

Observation period (years)	No of patients	Died of tumour	Died without evidence of tumour	Determinate survival rate		
				No of patients	No of survivals	Survival (per cent)
5	75	44	10	65	21	32
10	63	40	14	49	9	18
15	54	33	15	39	6	15
20	45	28	14	31	3	10
25	34	21	11	23	2	9

42 years The determinate survival rate (DSR) is calculated from the first histologic verification of the neoplasm All 75 patients were controlled for at least 5 years, 63 for at least 10 years, 54 for at least 15 years, 45 for at least 20 years and 34 patients for at least 25 years The determinate survival rate fell from 32 for the patients followed up for 5 years to 9 per cent for the 25-year group (Table 5)

Forty-eight patients with parotid tumours died These were made up of 27 patients who died within 5 years of the first symptoms, 11 between 5 and 10 years, 3 between 10 and 15 years, 1 between 15 and 20 years and 4 patients after more than 20 years The interval was uncertain in 2 patients

Discussion

Since many of the neoplasms had been irradiated before operation, the histologic structure might have undergone such alteration as to affect the classification Although it is difficult to assess the importance of this factor, it was not felt to constitute a major problem Some of the growths, however, presented marked retrogressive change, probably as a result of the irradiation

Highly malignant, poorly differentiated tumours of the parotid gland are fortunately not common The present material comprising 1 678 parotid tumours consisted of 299 (18 per cent) classified as malignant with 75 of these as poorly differentiated solid carcinomas In modern classifications the various types of salivary gland neoplasms are well defined on the basis of differentiation of the histologic structures (Blanck et coll 1967, 1971, Eneroth et coll 1966, 1968 Jakobsson et coll 1968), the poorly differentiated solid carcinoma being best characterized by absence of differentiation The variation in incidence of poorly differentiated solid carcinoma in the literature may be explained by

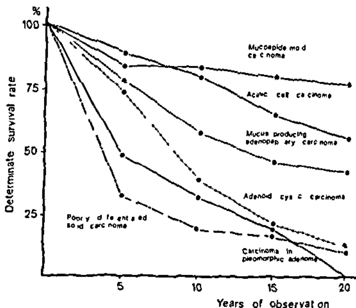


Fig 6 Malignant parotid tumours Relation between duration of observation and determinate survival rate

the fact that the classification of a tumour rests on its most differentiated part As other types of salivary gland malignancies may contain poorly differentiated as well as more differentiated areas, there is a risk that such a type will be classified as poorly differentiated carcinoma if the histologic analysis has been based on a specimen containing only poorly differentiated structures The more differentiated structure may be overlooked if the specimens available are too small, because of the high incidence of minor surgical interventions this must sometimes have occurred in the present investigation

Carcinoma in pleomorphic adenoma (malignant mixed tumour), adenoid cystic carcinoma and mucoepidermoid carcinoma often contain areas of poor histologic differentiation The present investigation comprised 1 678 parotid tumours treated in 1909 to 1958, to which ENEROTH (1971) added a material of 480 parotid tumours treated by parotidectomy during a 10-year period, 1959 to 1969 In this later material in which the whole tumour was available for histologic examination the incidence of poorly differentiated solid carcinoma was 1 9 as compared to 4 5 per cent in the present report The incidence of poorly differentiated solid carcinoma in this latter is probably too high, due to the possible omission of more differentiated histologic structures typical of better defined types of neoplasm

Poorly differentiated solid carcinomas run the most malignant course of all malignant parotid tumours A comparison of the determinate survival rate after 5, 10, 15 and 20 years is made between the different types of malignant tumour in Fig 6

It is apparent that the prognosis for poorly differentiated solid carcinoma is much worse than for the other types, apart from carcinoma in pleomorphic adenoma The difference in prognosis is especially remarkable at the 5-year control when the DSR is between 84 and 73 per cent in mucoepidermoid carcinomas (JAKOBSSON et coll 1968), acinic cell carcinoma (ENEROTH et coll 1966) mucus-producing adenopapillary carcinoma (BLANCK et coll 1971) and adenoid cystic carcinoma (BLANCK et coll 1967), whereas the 5-year DSR of poorly differentiated solid carcinoma is 32 per cent Even after 10, 15 and 20 years a distinct difference exists in the prognosis between poorly differentiated solid carcinoma and other types of tumour The exception is carcinoma in pleomorphic adenoma and adenoid cystic carcinoma at the 20-year control, the latter type being characterized by a prolonged clinical course with a grave long term prognosis (BLANCK et coll 1967)

The similar prognosis between poorly differentiated solid carcinoma and carcinoma in pleomorphic adenoma may be explained by the malignant component in the latter type, usually consisting of poorly differentiated solid carcinoma, poorly differentiated adenoid cystic carcinoma or poorly differentiated mucoepidermoid carcinoma (MOBERGER & ENEROTH 1968) It thus becomes obvious that the prognosis for other types of neoplasm with areas of poorly differentiated histologic structures seems to be as bad as for the poorly differentiated solid carcinoma itself

The present investigation has revealed that peri and intraneural as well as intravascular invasion is a grave prognostic sign, whereas it has been difficult to unravel any definite clues as to the prognosis from other histologic features

All patients with a poorly differentiated solid carcinoma and concomitant spontaneous facial nerve paralysis died regardless of the radical nature of the surgery The investigation of 46 patients with spontaneous facial nerve paralysis caused by different malignant conditions disclosed that invasion of the facial nerve seems to defy present therapeutic procedures (ENEROTH 1972) The similarity between the incidence of spontaneous facial nerve paralysis and the impairment in the prognosis was demonstrated by a correlation between the presence of this sign and the DSR of the different malignant parotid tumours (ENEROTH 1972) The incidence of facial nerve paralysis was thus definitely higher in high grade malignant conditions like adenoid cystic carcinoma and poorly differentiated solid carcinoma than in the lower grade malignant types such as mucoepidermoid and acinic cell carcinoma The same research also disclosed that the survival time after the onset of paralysis was strikingly short in high grade malignant conditions, i e poorly differentiated solid carcinoma compared to the lower grade malignant types

No definite conclusions could be drawn about the prognosis from other clinical features such as the size of the tumour and its attachment and hard consistency as well as dermal ulceration, although these factors seem to imply a worsening of the prognosis The difficulty in finding any conclusive prognostic clues from most histologic and clinical features may have been due to the fact that the overall prognosis is so bad in the tumour group as a whole as to make it difficult to divide the material into groups with different prognoses The long period covered by the material makes an evaluation of different forms of the varying therapeutic measures, however, of limited value

It was not until the late 1950s that the treatment became more uniform Thus as the poorly differentiated solid carcinoma has proved to be a relatively sensitive tumour, nowadays irradiation is given before surgery as a routine Total parotidectomy with sacrifice of the facial nerve and radical neck dissection are also now recommended for this type of neoplasm (ENEROTH 1973)

The poor prognosis that has been reported may now be said to have been supplanted by improvement expected from more adequate treatment

The investigation indicated that the prognosis of other better defined types of tumour was impaired and the same as poorly differentiated solid carcinoma if these contained areas of similar histologic structure It was also evident that poorly differentiated solid carcinomas caused a high incidence of spontaneous facial nerve paralysis, were more commonly ulcerated, had a higher incidence *of metastases and a lower survival rate than the other malignant types*

SUMMARY

A reclassification of 1 678 parotid tumours indicated that 75 (4 5 per cent) were poorly differentiated solid carcinomas Spontaneous facial paralysis and metastatic spread were common and the determinate survival rate at 5 years was lower than in any other malignant growth of the parotid gland Preoperative irradiation total parotidectomy with sacrifice of the facial nerve and radical neck dissection appear to provide a means of improving the poor prognosis

ZUSAMMENFASSUNG

Eine Reklassifizierung von 1 678 Parotistumoren zeigte dass 75 (4 5 Prozent) niedrig differenzierte solide Karzinome waren Eine spontane Facialis Paralyse und metastatische Streuung waren gewohnlich und die festgestellte 5 Jahres Überlebensrate war niedriger als bei jedem anderen malignen Wachstum der Parotis Druse Praoperative Bestrahlung totale Parotidektomie unter Opferung des Nervus facialis und radikale Dissektion des Halses scheinen eine Moglichkeit zu bieten die schlechte Prognose zu verbessern

RÉSUMÉ

Un reclassement de 1 678 tumeurs parotidiennes a montre que 75 tumeurs (4,5 pour cent) etaient des carcinomes solides peu differencies La paralysie faciale spontanee et la dissemination metastatique sont frequentes et le taux de survie definitif a 5 ans est plus bas que dans aucune autre tumeur maligne de la glande parotide L irradiation pre operatoire la parotidectomie totale avec sacrifice du nerf facial et dissection cervicale radicale paraissent fournir le moyen d améliorer ce mauvais pronostic

REFERENCES

BLANCK C ENEROTH C M and JAKOBSSON P Å Mucus producing adenopapillary carcinoma of the parotid gland Cancer 28 (1971), 676

— — and JACOBSSON F Adenoid cystic carcinoma of the parotid gland Acta radiol Ther Phys Biol 6 (1967) 177

ENEROTH C M Histological and clinical aspects of parotid tumours Acta oto-laryng (Stockh) (1964) Suppl No 191

— Salivary gland tumors in the parotid gland submandibular gland and the palate region Cancer 27 (1971), 1415

— Facial nerve paralysis a criterion of malignancy in parotid tumors Arch Otolaryng 95 (1972), 300

— Parotid tumours and the facial nerve *In* Chirurgie des Nervus facialis Herausgegeben von N Miehlke Urban & Schwarzenberg Munchen und Berlin 1973

— JAKOBSSON P Å and BLANCK C Acinic cell carcinoma of the parotid gland Cancer 19 (1966) 1761

— BLANCK C and JAKOBSSON P Å Carcinoma in pleomorphic adenoma of the parotid gland Acta oto-laryng (Stockh) 66 (1968) 477

FOOTE F W JR and FRAZELL E L Tumors of the major salivary glands *In* Atlas of tumor pathology Sect IV fasc 11 Armed Forces Institute of Pathology, Washington D C 1954

JAKOBSSON P Å BLANCK C and ENEROTH C M Mucoepidermoid carcinoma of the parotid gland Cancer 22 (1968) 111

MOBERGER G and ENEROTH C M Malignant mixed tumors of the major salivary glands Cancer 21 (1968) 1198

RAUCH S Die Speicheldrusen des Menschen Thieme Verlag Stuttgart 1959

WALSTAM R Studies on therapeutic short-distance and intracavitary gamma beam techniques Acta radiol (1965) Suppl No 236

FROM THE INSTITUTE OF RADIATION PHYSICS, AND THE DEPARTMENT OF TUMOUR BIOLOGY, KAROLINSKA INSTITUTET, 104 01 STOCKHOLM, SWEDEN

DOSE FROM SCATTERED ROENTGEN RADIATION TO A PROTECTED PART OF A MOUSE PHANTOM

I BOJTOR, K HIESCHE and P O SCHNELL

Partial shielding of the animal organism exposed to roentgen radiation is frequently used in experimental investigations into the radiation effect on the hemopoietic system or the organs of immunologic importance Mice are often used in such research, a leg or part of a leg being covered by lead shields of various shapes Although efficient protection arrangements may be made against the primary radiation, the volume shielded may still be exposed to scattered radiation of considerable biologic effects

Several authors have referred to the scattered radiation in a protected part of the body (HANKS 1964, DE VRIES & VOS 1966, FUJIOKA et coll 1967, BLOMGREN & REVESZ 1968, GIDALI et coll 1969, DAVIS & COLE 1969, CROIZAT et coll 1970, FRIED et coll 1971, CARSTEN & CRONKITE 1971, RAUCHWERGER 1972) A thorough investigation of such radiation in the shielded thymic region of the rat was made by CORP & MOLE (1961) with a rat phantom and ionization chamber method The purpose of the present investigation was to report on determinations of the dose from scattered radiation in the mouse leg covered by lead shields of various shapes

Submitted for publication 22 January 1973

Method The experiments were carried out with plane parallel phantoms consisting of a body and a leg of a size corresponding to the volume of the adult mouse Three phantoms were applied in the same pattern as in animal experiments, i e radially on a circular plexiglass plate at an angle of 20° with the leg turned outwards The measurements were made in the central phantom, the two lateral phantoms being so applied as to allow for the lateral scattering that may occur at standard irradiation procedures, the centre of the phantoms was placed in the centre of a radiation beam of 40 cm diameter A Philips integrating dosimeter lay on the surface of the central mouse phantom (Fig 1) Irradiation factors 200 kV roentgen, 1 mm Cu HVL and 78 cm SSD The dose measurements were performed with 6 mm LiF Teflon TL rods of 1 mm diameter placed in the central phantom, three rods were positioned at 0 4 cm depth in the phantom body and four in the leg at 1 4, 2, 2 6, 3 2 cm from the body The purpose of this positioning of the rods at 0 4 cm depth was to ensure an appropriate region for both the electronic equilibrium and the backscatter It was therefore possible to express the TL responses in rad at the calibrations and at the measurements by the method given by MÅRTENSSON (1969) The rods had individually been calibrated for the roentgen qualities that occurred in the present experiments Ten repeat calibrations indicated that the standard deviation of the response of the different rods was ± 2 5 per cent maximum No change in the individual correcting factors was necessary to allow for different radiation qualities

The scattered radiation in the phantom leg was measured with four different shielding arrangements 0 45 cm thick lead in shape of a rhombus (area 9 cm^2), a tunnel (4 cm long), an open-ended cylinder (inner ϕ 0 8 cm, 1 6 cm long) or a close-ended cylinder (inner ϕ 0 8 cm, 5 2 cm long) When the rhomboid shield was used a 2 cm × 1 2 cm piece of lead was placed under the leg to minimize backscatter from the plexiglass plate

Results

The percentage dose from scattered radiation in the phantom leg protected by any of the four different arrangements was determined by at least five repeat measurements and the mean calculated The converting factor from R to rad valid for muscle (JOHNS & CUNNINGHAM 1969) was employed to estimate the absorbed dose, due to the relatively small size of the phantom, this conversion necessarily implies some inaccuracy In view of the different energy of the scattered photons reaching the leg as compared to those in the body (BRUCE & JOHNS 1960) the response of the LiF dosimeter in the former was corrected by 6 per cent to take into account the energy spectra of scattered photons at 2 cm

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Conclusions

The measurements indicate that a great deal of scattered radiation may reach the protected parts of the leg of a mouse depending on the shielding arrangements This radiation may be of important biologic significance, as stated by CORP et coll , and must therefore be taken into consideration

Acknowledgements

The authors wish to thank Prof Rune Walstam and Prof Laszlo Revesz for their advice and help

SUMMARY

The dose contribution from scattered radiation to a shielded region of a leg in a mouse phantom was determined under specified conditions It proved to vary between 0 2 and 8 per cent of the maximum delivered to the phantom body

ZUSAMMENFASSUNG

Die Dosis durch Streustrahlung in einem geschutzten Teil des Beines eines Mausephantoms wurde unter bestimmten Bedingungen gemessen Die Dosis variierte zwischen 0 2 und 8 % der maximalen Dosis mit der das Phantom bestrahlt worden war

RÉSUMÉ

La contribution de dose due au rayonnement diffuse a une region d une patte d un fantome de souris protegee par un ecran contre les radiations a ete determinee dans des conditions specifiques Cette contribution varie entre 0 2 et 8 % du maximum delivre au corps fantôme

REFERENCES

BLOMGREN H and RÉVÉSZ L The effect of bone marrow protection on the cellular composition of irradiated thymus Exp cell Res 53 (1968), 261

BRUCE W R and JOHNS H E The spectra of X rays scattered in low atomic number materials Suppl No 9 p 40 The British Inst of Radiology, London 1960

CARSTEN A L and CRONKITE E P Comparison of autologous marrow injection to shielding in lethal irradiation of the mouse Proc Soc exp Biol Med 137 (1971), 948

CORP M J and MOLE R H Abdominal irradiation and the thymus of the adult rat the relative importance of the adrenal glands and of scattered radiation Amer J Roentgenol 85 (1961) 550

CROIZAT H FRINDEL E and TUBIANA M Proliferative activity of the stem cells in the bone marrow of mice after single and multiple irradiations (total or partial body exposure) Int J radiat Biol 18 (1970), 347

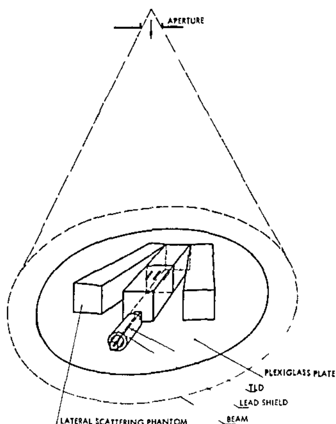


Fig 1

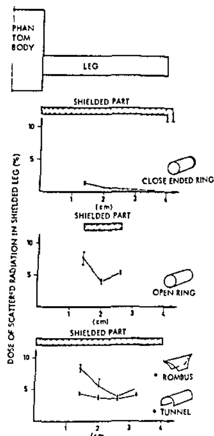


Fig 2

Fig 1 Experimental arrangement The phantoms were exposed to 400, 700, 1 000 R with the leg of the phantom protected by lead shields of various shapes

Fig 2 The dose measured from scattered radiation in the phantom leg as a percentage of the absorbed dose at 0 4 cm depth in the phantom body The mean and the variance calculated from at least five measurements are indicated

depth from incident roentgen radiation of 80 to 90 keV E $_{ff}$ Figure 2 illustrates the dose to the leg at various distances from the body as a percentage of the dose absorbed at 0 4 cm depth A significant variation in this dose from scattered radiation may be observed at different positions in the leg The maximum dose was obtained at that part of the leg nearest the body in each experiment, this was about 8 per cent at the rhomboid-shaped lead shield and at the open-ended cylinder The dose in both instances also increased with the distance from the middle of the leg where it was minimal It was apparent that the dose varied greatly with the shielding arrangement, greater efficiency is obtained with a close-ended cylinder or a tunnel

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RADIATION QUALITY INDEPENDENT LIQUID IONIZATION CHAMBER FOR DOSIMETRY OF ELECTRON RADIATION FROM MEDICAL ACCELERATORS

GÖRAN WICKMAN

For the determination of absorbed dose and dose distribution with high energy electrons in tissue like materials, the use of air filled thimble ionization chambers in water is usually recommended (HPA 1971, ICRU 1972, NACP 1972) The advantages of these detectors lie mainly in their good precision Readings can be made in rapid succession and the results are immediately available However, due to the different polarization effects in the gas and the surrounding medium, the absorbed dose calibration factor for this type of detector depends both on the initial energy of the electrons and the depths of the probe in the irradiated object (HARDER 1965, SVENSSON & PETTERSSON 1967)

Due to the large difference in the density of the water and detecting gas, [illegible] 1968), i e the [illegible] interest If the [illegible] is thimble shaped, a displacement correction must also be applied (DUTREIX & DUTREIX 1966, HETTINGER et coll 1967) The corrections mentioned depend on the energy of the electrons and their angular distribution at

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DAVIS W E and COLE L J Retarded immunological recovery in sublethally X irradiated mice by additional thymic exposure Reversal with injected marrow cells Proc Soc exp Biol Med 130 (1969), 1336

FRIED W KNOSPE W H, GREGORY S A and TROBAUGH JR F F Factors regulating the proliferation and migration of hematopoietic stem cells J Lab Clin Med 77 (1971) 239

FUJIOKA S HIRASHIMA K, KUMATORI T TAKAKU F and NAKAO K Mechanism of hematopoietic recovery in the X irradiated mouse with spleen or one leg shielded Radiat Res 31 (1967), 826

GIDÁLI J FEHÉR J GALLYAS A and VÁRTERÉSZ V Colony forming cells in irradiated partially shielded endotoxin treated mice Radiat Res 39 (1969), 305

HANKS G E In vivo migration of colony forming units from shielded bone marrow in the irradiated mouse Nature 203 (1964) 1393

JOHNS H E and CUNNINGHAM J R The physics of radiology Third edition p 736 Charles C Thomas, Springfield 1969

MÅRTENSSON B K A Thermoluminescence of LiF A statistical analysis of the influence of pre annealing on the precision of measurement Phys in med Biol 14 (1969) 119

RAUCHWERGER J M Radiation protection by tibia shielding in adult weanling and suckling mice Int J radiat Biol 22 (1972), 269

DE VRIES F A J and VOS O Prevention of the bone marrow syndrome in irradiated *mice* A comparison of the results after bone marrow shielding and bone marrow inoculation Int J radiat Biol 11 (1966), 235

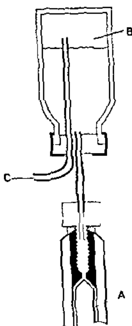


Fig 2 The pressure on the chamber cavity A and the liquid con

Ionization chambers and charge measurement equipment Thimble ionization chambers (Siemens Sondenfingerhut) were used as reference monitors and for absorbed dose determinations The charge measurements were carried out using automatically compensated Townsend Balance equipment constructed at the laboratory (WICKMAN 1969)

Chamber construction

The main parts of the chamber are made of a styrene copolymer (Rexolite 1422), consisting mainly of polystyrene (density $\rho = 1.05$ g cm^{-3}) The dielectric properties of this material are comparable to those of amber and are not seriously affected by radiation (WICKMAN 1972)

In order to make ion collection possible, semi transparent films of beryllium have been evaporated on the insulator material While beryllium is toxic, particularly to the lungs, which made the evaporation more complicated, this material was considered as the most suitable The material of the chamber cavity must not react chemically with the liquid and 'poison' it, causing the leakage to increase These effects caused major difficulties when, for example, aluminium of high purity was employed The chamber showed energy dependence when a heavy

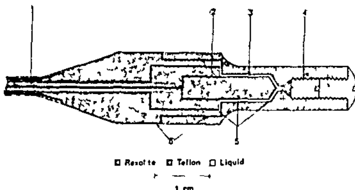


Fig 1 Cross sectional view of the chamber 1—Teflon insulated low noise triaxial cable 2—Guard electrode 3—Central electrode 4—Filling valve 5—Evaporated, thin layers of Be 6—High voltage electrode

the measuring point Consequently, accurate dose determinations can be made only when these quantities are known In practice this means that accurate absorbed dose measurements are limited to the central beam in homogeneous water phantoms These negative effects can be eliminated if the ionization is measured in a condensed medium instead of a gas Most liquid hydrocarbons have such properties with regard to conductivity and ion mobility that it is possible to obtain the advantages of conventional ionization chambers and avoid the disadvantages listed above

Since THOMSON (1895) discovered the ionization in dielectric liquids irradiated with roentgen rays, little has been published on methods for using a liquid ionization chamber as a field instrument for dosimetry MATHIEU et coll (1969) and CASANOVAS et coll (1971) have reported success in the use of liquid ionization chambers with electron radiation However, the construction of these chambers and their lack of spatial resolution limit practical applications In the present report, the fundamental properties of an ionization chamber prototype are presented The chamber is intended for use as a routine instrument for absorbed dose determinations in electron radiation fields of medical accelerators

Materials

Radiation sources Temperature dependence, reproducibility and calibration stability have been tested with a 25 Ci ^{60}Co radiation source The chamber was placed in a lucite fixture in order to obtain reproducible geometry The exposure rate was approximately 25 R min^{-1} A 5 kCi ^{60}Co radiation source (Siemens Gammatron I) was used for the investigation of the effects of irradiation on the chamber The exposure rate at the chamber was approximately 1 kR min^{-1}

The properties of the chamber have been determined in the electron radiation field from a Brown Boveri 35 MeV betatron with a modified scattering foil and collimator system (SVENSSON 1971)

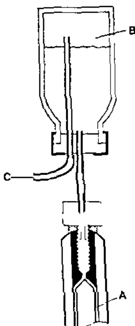


Fig 2 The pressure on the chamber cavity A and the liquid container B is decreased by pumping at C When the pump is disconnected, the chamber cavity is filled with liquid Remaining air in the cavity is efficiently washed away by a repeated pumping to such a low pressure that the liquid starts to boil

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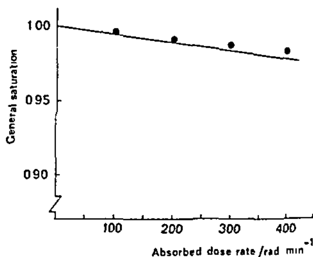


Fig 3 General saturation at pulsed radiation (pulse length ≈ 10 μs frequency 50 s⁻¹) Points denote experimental data, solid line theory

inert metal, e g gold, was used despite the thinness of the metal films The sensitive part of the chamber consists of a tube-shaped layer of liquid with the following dimensions length 10 5 mm, average radius 2 2 mm and thickness 0 3 mm The layer of liquid was made very thin in order to achieve high field strength and short ion travel, which allow a small volume recombination with pulsed radiation at dose rates of a few hundred rad min⁻¹ The liquid used was 2,2,4-trimetylpentane (Merck, isooctan fur die Spektroskopie)

Chamber performance

The residual current of the liquid, choice of polarization voltage With no purification of the commercially available liquid and after careful washing of the chamber cavity, a resistivity of 10^{16}—10^{17} Ωcm at 20° C was obtained for chamber voltages up to 900 V corresponding to an average field strength of 2 7 MV m⁻¹ This is approximately one tenth of the values presented for extremely pure liquids (Schmidt 1968, Tewari & Freeman 1968, Mathieu 1968) At higher chamber voltages, the current is no longer proportional to the voltage applied by Charalambus (1967) and a decline of the resistivity results This effect has been investigated in detail and was assumed to depend on electron emission from the cathode (Schottky effect) or electrolytic dissociation of the liquid molecules Therefore, chamber voltages above 900 V were not ordinarily used with the present chamber At 900 V polarization voltage and 20° C, the residual current with fresh liquid in the chamber is equivalent to the ionization current produced by an absorbed dose rate of 0 1 rad min⁻¹ The relative contribution from the residual current to the total current can be determined with a precision better than 10 per cent with the charge measurement equipment

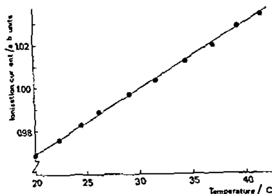


Fig 4 Ionization current at constant exposure rate and different temperatures Normalized to current at 30° C

used This means that, in determining the dose rate, the error caused by the residual current is estimated to be less than ± 0 01 rad min^{-1} In most cases, this error is negligible compared with the total variation of the fluence rates in electron radiation fields of medical accelerators

General saturation In order to obtain a precision equal to that of conventional ionization chambers, the general recombination must not exceed a few per cent for pulsed radiation and mean dose rates as large as 500 rad min^{-1}, which is normal for medical accelerators

In general, if space charge effects are ignored, the fraction f of the ions actually reaching the electrodes when a voltage V is applied to a homogeneously ionized medium containing n_0 ion pairs per cm^3 in a parallel plate chamber of plate separation d is given by

$$f = u^{-1}\ ln\,(1+u)$$

$$\text{where}\quad u = \frac{n_0\ \alpha\ d^2}{\mu\ V},\quad \text{(Boag 1950)}$$

μ is the mobility of the ions in cm s^{-1} and α the recombination coefficient With nonpolar liquids the ratio of recombination to mobility is given by

$$\frac{\alpha}{\mu} = \frac{e}{\varepsilon_r\ \varepsilon_0}\quad \text{(Debye 1942)}$$

where e = electron charge, ε_r = relative permittivity of the liquid and ε_0 = the permittivity of vacuum (SI units) Calculation using the above formulas with $d = 3 \times 10$ cm, V = 900 V, and $\varepsilon = 1\ 94$ (20° C) results in an $f > 0\ 95$ for absorbed dose rates $\leqslant$ 1 000 rad min^{-1} The experimental and theoretical values

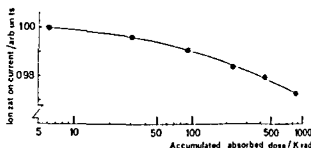


Fig 5 Ionization current at constant exposure and different accumulated absorbed doses Normalized to current in fresh liquid

for f are presented in Fig 3 No correction was made for the geometry of the chamber since the ratio between the inner and outer radius of the liquid cylinder ≈ 1

Temperature dependence The dependence of the ionization current upon temperature was analysed in the temperature range 20 to 40° C using ^{60}Co γ-radiation The current increases linearly by 0 3 per cent per ° C (Fig 4) The change in the current is due mainly to the fact that initial recombination decreases at high temperatures ONSAGER (1938) demonstrated that, in gases with high pressure and no applied outer field, the probability p of the escape of an electron that has reached thermal equilibrium and formed a negative ion at a distance r from the parent positive ion is given by

$$p = \exp(-r_c/r)$$

where r_c is the critical distance between the ions when the potential energy of the ion pair is equal to the thermal $K \times T$ or

$$r_c = \frac{e^2}{\varepsilon_r \ \varepsilon_0 \ K \ T}$$

The applied electric field causes an increase in p but, as approximately half the number of the ions formed still recombine by means of initial and columnar recombination, the temperature effect cannot be neglected

Precision The standard deviation of repeated readings using ^{60}Co γ-irradiation (25 R m^{-1}) with a chamber voltage of 900 V was found to be less than 0 2 per cent This value includes the errors in charge measurement equipment and exposure time

Calibration stability The sensitivity of the chamber, Q/X, where Q is the net charge collected following an exposure X in a ^{60}Co γ-ray beam, shows a slight dependence on the age and the radiation history of the ionization liquid The variation of Q/X as a function of age is most evident immediately after the chamber has been filled with fresh liquid, when it reaches a maximum of

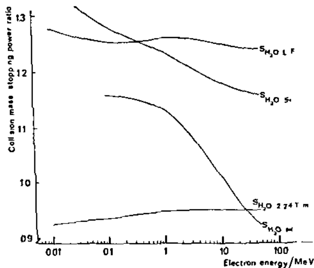


Fig 6 Computed collision mass stopping power ratios for electrons

approximately —1 per cent per week The change in sensitivity caused by the radiation history of the liquid has been investigated with ^{60}Co ; radiation at an exposure rate of 1 kR min $^{-1}$ and was found to be approximately —1 per cent per 10^5R (Fig 5) At the same time, the residual current increases with age No detailed test has been made with respect to the residual current However, experience has shown that the chamber should be refilled after 10^4R or approximately once a week if a residual current twice the current of a recently filled chamber can be accepted

The change of the conductivity of the chamber probably depends on radiolysis and contamination due to chemical interactions between the liquid and the wall material of the chamber The reduction of sensitivity with increased contamination may depend on the fact that it serves as an electron scavenger with the result that the electrons travel a shorter distance from their parent ions before becoming localized This shorter ion separation distance causes increased initial recombination A decrease in the ionization current has earlier been observed when a small amount of an electron scavenger has been added to hydrocarbons (TEWARI & FREEMAN 1968) When the chamber is filled with fresh liquid, the original calibration value is obtained once again The sensitivity of the chamber has been tested after each of 20 changes of liquid The maximum deviation from the average value was less than ± 0 5 per cent In this investigation, liquids from several different batches were used

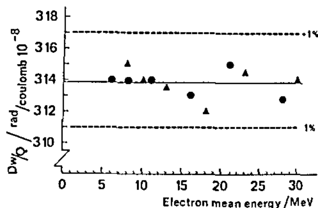


Fig 7 The absorbed dose calibration factor D_c/Q at different electron mean energies and depths in the phantom, z ▲ z = 1 cm ● z = 2 cm

Quality dependence

The probe has an average density of 1 04 g cm $^{-3}$ and consists purely of materials with low atomic numbers (H, Be and C) Therefore, in measurements in water, the disturbance of the electron fluence at the point of interest can be expected to be very small The principal factor that might cause changes in the absorbed dose calibration of a chamber is the variation of the mass collision stopping power ratio $S_{w,i}$ (w = water, i = probe sensitive material), with electron energy Since it is difficult to determine the electron energy distribution at an arbitrary point in an irradiated object, $S_{w,i}$ for an ideal detector material *should be independent of the electron energy*

The collision mass stopping power ratio for water to 2,2,4-trimetylpentane has been calculated from tabulated values of $(S/\varrho)_{col}$ for water, carbon and liquid hydrogen from BERGER & SELTZER (1964, 1966) (Fig 6)

Calculations by BERGER & SELTZER (1969 a, b) show that the secondary electrons with energies below 0 1 MeV make a constant contribution to the absorbed dose and have an energy distribution which remains approximately constant with increasing depth and incident energies About 25 per cent of the total absorbed dose is produced by these electrons and 70 per cent by electrons with energies greater than 1 MeV As a result, the S_{r} variation for electron energies above 1 MeV is of principal interest in estimating the constancy of the absorbed dose calibration of a detector The variation of $S_{r,i}$ for 2,2,4-trimetyl pentane in the energy range 1 to 50 MeV is 0 2 per cent while the deviations for two other alternative condensed detector materials proposed by ICRU, LiF and Si, in the same energy range are 2 and 7 per cent, respectively

As the columnar recombination of the liquid cannot be neglected at the field strength normally used (2 7 MV m $^{-1}$) the ionization density is important to the *collection efficiency* The signal, *normalized* to absorbed dose in the detector material, should therefore decrease with increasing LET This effect is of no practical importance in dosimetry for radiation fields of high energy electrons as

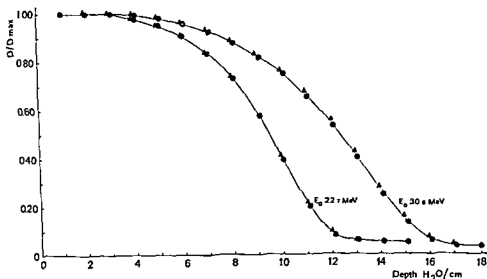


Fig 8 Relative depth dose distribution for electrons with initial energies E_0 of 22 7 MeV and E_0 of 30 6 MeV ● $FeSO_4$ measurements ▲ liquid ionization measurements

LET is practically constant regardless of depth and initial energy (BERGER & SELTZER 1969 b)

Comparison with air ionization chamber dosimetry The constancy of the absorbed dose calibration factor D_w/Q at different electron energies was investigated in the central ray at 1 and 2 cm depth in a water phantom irradiated with electrons with initial energies of 8 to 32 MeV The absorbed dose at the point of interest at a given monitor deflection was determined through ionization measurements with a Siemens Sondenfingerhutkammer The absorbed dose was calculated from the ionization measurement with the corrections for displacement and pertubation and the C_E values given by ICRU (1972) The air ionization chamber was then replaced by the liquid ionization chamber and the irradiation repeated

The values for D_w/Q at different depths and electron mean energies are shown in Fig 7 These results show that the absorbed dose calibration factor is practically independent of the electron mean energy in the region investigated

Comparison with $FeSO_4$ dosimetry Displacement effects and changes in the response of the liquid chamber with variation in the angular and energy distribution of the electrons were investigated and compared to those with $FeSO_4$ dosimetry

The measurements were made in the central beam in a 30 cm × 30 cm × 30 cm water phantom irradiated with electrons with initial energies E_0 of 22 7 and 30 6 MeV field size 10 cm × 10 cm and SSD 100 cm Depth dose measurements with $FeSO_4$ dosimetry were carried out with $FeSO_4$ cells placed in succession along the central beam according to a technique described by PETTERSSON & HETTINGER (1967) In ionization measurements with the liquid chamber and the same electron energies and radiation geometry the geometrical centre of the liquid chamber was considered to be the measurement point and corrections were made for variations in general recombination only The results (Fig 8) show that the differences between the relative depth dose measured with $FeSO_4$ and liquid ionization were small and non systematic Thus the absorbed dose calibration of the ionization chamber appears to be as constant as that of $FeSO_4$ over a wide range of measurement depths and with two initial energies No significant difference in extrapolated range for the electrons was observed and consequently no displacement effects occur

Discussion

As a complement to other systems recommended for dosimetry with high energy electron radiation the liquid filled ionization chamber represents an alternative with valuable advantages and few drawbacks The most important advantage is the fact that the absorbed dose calibration is energy independent to the same extent as $FeSO_4$ dosimeters while the sensitivity is considerably higher The precision of the liquid chamber equals that of $FeSO_4$ dosimeters in absorbed dose measurements at approximately 1/1 000 of the dose The simple measurement equipment used for conventional ionization chambers is all that is needed for the liquid chamber Since the ionization current in the liquid chamber is approximately 300 times that in a conventional ionization chamber of the same volume the sensitive volume of the liquid chamber can be made very small This is of great value in build up measurements and dose measurements near density inhomogeneities in irradiated objects

Another interesting property of the liquid ionization chamber is that an estimate of the LET of the radiation can be obtained by measuring the variation of the columnar recombination as a function of the field strength in the liquid (BLANC et coll 1963 CHARALAMBUS 1967) An LET detector with good spatial resolution is valuable in plotting the LET in fields for radiation therapy since not only the distribution of absorbed dose but also variations in the microdosimetric energy deposition in the radiation field are important to the biologic effect

The present detector has been used for LET investigations in ^{60}Co- and electron radiation fields and the results show good resolution, reproducibility and conformity to theoretical calculations The results will be published in the near future

Acknowledgement

The author is greatly indebted to Mr Thord Holmstrom for constructional work necessary for this project This investigation was supported by the Swedish Cancer Society (Project No 466)

SUMMARY

A cylindrical ionization chamber with a layer of 0 3 mm 2 2 4 trimetylpentane used as the ionization volume has been constructed The dosimetric properties of the detector used in electron radiation fields from medical accelerators have been investigated

ZUSAMMENFASSUNG

Es wurde eine zylinderformige Ionisationskammer mit einer 0 3 mm starken Schicht von 2 2 4 Trimethylpentan die als Ionisationsvolumen verwendet wurde konstruiert Die dosimetrischen Eigenschaften des Detektors der bei Elektronen Strahlen Feldern von medizinischen Acceleratoren verwendet wurde ist untersucht worden

RÉSUMÉ

L auteur a construit une chambre d ionisation cylindrique avec une couche de 0,3 mm de 2,2 4 trimethylpentane utilisee comme volume d ionisation Il a etudié les propriétes dosimetriques de ce detecteur utilise dans des champs de radiation d electrons provenant d accelerateurs medicaux

REFERENCES

BERGER M J and SELTZER S M Tables of energy losses and ranges of electrons and positrons NASA SP 3012 National Aeronautics and Space Administration Washington D C ,1964

— — Additional stopping power and range tables for protons, mesons and electrons. NASA SP 3036 National Aeronautics and Space Administration Washington D C., 1966

— — a) Calculation of energy and charge deposition and of the electron flux *in a water* medium bombarded with 20 MeV electrons *In* High-energy radiation therapy dosimetry Edited by J S Laughlin Ann N Y Acad Sci *161 (1969), 8*

— — *b) Quality of radiation in a water medium irradiated with high-energy electron* beams *In* Book of Abstracts p 127 12th International Congress of Radiology, Tokyo 1969

BLANC D MATHIE J et TORRES J P [illegible]
d ion [illegible]
appli [illegible]
Instr [illegible]

BOAG J W Ionization measurements at very high intensities Brit J Radiol 23 (1950), 601.

CASANOVAS J, PATAU J P, MATHIEU J et BLANC D Comparaison entre l ionisation produite dans le volume sensible d'une chambre d'ionisation a diélectrique liquide et l énergie qui y est déposée, calculée par la méthode de Monte Carlo, dans le cas d irradiation par des électrons monocinétiques de 1 5 et 1 9 MeV *In* Third symposium on microdosimetry Stresa, Italy, EUR 4810 d f e, p 571, 1971

CHARALAMBUS S The response of a liquid ionization chamber to gamma radiation and high energy neutrons and protons Nucl Instr Meth 48 (1967), 181

DEBYE P Reaction rates in ionic solutions Trans electrochem Soc 82 (1942), 265

DUTREIX J et DUTREIX A Étude comparée d'une série de chambres d ionisation dans des faisceaux d électrons de 20 et 10 MeV Biophysik 3 (1966), 249

HARDER D Berechnung der Energiedosis aus Ionisations Messungen bei Sekundärelektronen Gleichgewicht *In* Symposium on high energy electrons Montreaux 1964 Edited by A Zuppinger and G Poretti Springer Verlag, Berlin 1965

— The effect of multiple electron scattering on the ionization in gas filled cavities Biophysik 5 (1968), 157

HETTINGER G, PETTERSSON C and SVENSSON H Displacement effect of thimble chambers exposed to a photon or electron beam from a betatron Acta radiol Ther Phys Biol 6 (1967), 61

HPA Report Series No 4 A practical Guide to Electron Dosimetry The Hospital Physicists Association, London 1971

ICRU Report 21 Radiation Dosimetry Electrons with initial energies between 1 and 50 MeV International Commission on Radiation Units and Measurements, Washington D C 1972

MATHIEU J Thèse de Doctorat ès Sciences 313 Toulouse, 1968

— BLANC D, CASANOVAS J, DUTREIX A, WAMBERSIE A et PRIGNOT M Mesure de la répartition de la dose déposée en profondeur dans un fantome de plexiglas irradié par un faisceau d électrons monocinétiques de 10 15 20 ou 30 MeV *In* Second symposium on microdosimetry, Stresa Italy, EUR 4452 d f e, p 437, 1969

NACP Recommendations of the Nordic Association of Clinical Physics Procedures in radiation therapy dosimetry with 5 to 50 MeV electrons and roentgen and gamma rays with maximum photon energies between 1 MeV and 50 MeV Acta radiol Ther Phys Biol 11 (1972), 603

ONSAGER L Initial recombination of ions Phys Rev 54 (1938) 554

PETTERSSON C and HETTINGER G Dosimetry of high energy electron radiation based on the ferrous sulphate dosimeter Acta radiol Ther Phys Biol 6 (1967) 160

SCHMIDT W F and ALLEN A O Yield of free ions in irradiated liquids, determination by clearing field J phys Chem 72 (1968), 3730

SVENSSON H Influence of scattering foils, transmission monitors and collimating system on the absorbed dose distribution from 10 to 35 MeV electron radiation Acta radiol Ther Phys Biol 10 (1971) 443

— and PETTERSSON C Absorbed dose calibration of thimble chambers with high energy electrons at different phantom depth Ark Fysik 34 (1967) 377

TEWARI P H and FREEMAN G R Dependence of radiation induced conductance of liquid hydrocarbons on molecular structure J chem Phys 49 (1968) 4394

THOMSON J J *The roentgen rays* Nature (Lond) 53 (1895) 377

WICKMAN G Townsendkopplad jonkammarutrustning med automatisk kompensering och digital utläsning (In Swedish) Nord Med 82 (1969) 1264

— Small gas tight ionization chambers for absorbed dose control during radiation therapy Acta radiol (1972) Suppl No 313 p 152

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PROBE CHECK OF COMPUTER DOSIMETRY FOR GYNECOLOGIC IMPLANT THERAPY

Y MARUYAMA, B HOSKINS, D E WREDE, G SOWELL and J R VAN NAGELL JR

The advent of computer dosimetry in clinical radiation therapy provides an immense amount of data regarding dose distribution For example, the dose around an implant can be specified in great detail and in 3-dimensional array from currently readily available computer resources If this technologic advance is to provide a general increase in therapeutic care, the output data must be reliable, accurate and dependable for the prescription of dose to reference points Moreover, if the development represents a significant advance to improve the results of irradiation it must be evident that greater optimization in dose distribution has meaningful applications beyond the accustomed procedures ordinarily used One such advance would potentially rest upon differential dosage, with greater dose to the tumor or tumor bearing region, and less dose to radiation sensitive normal structures lying in the irradiated field

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Gynecologic radioactive implants represent a system where such optimization would appear reasonably achievable. For example, reference dosage points in the paracervical triangle (e.g. Pt A) are easily assigned and by means of radiography precise localization of adjacent radiation sensitive structures is easily accomplished using contrast media in the rectum and lower sigmoid region and bladder (Fig. 1). A Foley bag placed in the bladder and contrast medium in the bladder and ureters will localize those structures. Direct dosimetry measurements have also been described (SIEVERT 1932). It has been advocated that individualized patient dosimetry to fit the anatomic situation is essential to optimal irradiation. LEDERMAN (1950), DUTREIX (1954), FLETCHER et coll. (1958) and KOTTMEIER & GRAY (1961) have advocated direct measurements of dosage with probes placed in the rectum and bladder. However, there was felt to be little correlation between dosage and complications.

Recently, computer dosimetry systems have been developed that provide enormous data at numerous points in the pelvis. It was the purpose of the present investigation to assess the accuracy and reproducibility of computer dosimetry by direct probe measurements taken in the pelvis at the time of implantation. We believe on the basis of our experiences that this system provides a reliable and accurate monitoring system to deliver more accurate patient dose.

Clinical material and procedures

The material consists of cases of pelvic implants done during the period of January 1, 1971 to July 1, 1972. The procedures followed aimed to place markers on the cervix, contrast medium in the rectum and lower sigmoid and bladder, and to use applicators or dummy sources so that the position of all vital structures and the irradiation sources relative to the application of sources could be accurately defined. A system was developed using right angle orthogonal films to record the positions of all the above structures.

To be described in this paper are the results of our investigations between this simulation system and direct determination by probe. In a Rando-phantom correlation by simulation and with the applicators loaded with sources was excellent (to be reported elsewhere).

Tandems and ovoids were positioned in the uterine canal and fornices of the vaginal vault respectively. Clips were placed on the cervix. The anterior rectal wall was coated with barium paste. Positive contrast medium was placed in the Foley bag and air into the bladder to define its position. Orthogonal films were obtained upon which axes were drawn and coordinates obtained for positions of all radioactive sources in the x, y, z axes. Using the IBM 360/65 computer system a complete analysis of each implant was possible to all relevant points



Fig 1 Contrast medium in bladder and rectum Detecting probe is placed in rectum

e g Pt A B, bladder, rectum, sigmoid colon, vaginal surface, etc A coin was placed in the vaginal vault to obtain accurate magnification factors

A Siemens cadmium sulfide probe inside a rubber sheath was also routinely positioned in the rectum at the time when the films were obtained The probe position was also recorded and localized similarly to all other points in the pelvis Our initial efforts were to obtain multiple readings along the anterior rectal wall of the bladder However, it soon was apparent that a single accurately localized position would suffice to monitor the dosimetry for the entire implant Thus, our method evolved from numerous readings at approximately 1 cm axial rectal positions, at unknown distances from the radium system, to a single careful reading at an accurately known position and distance from the radium system

Correlation of computer dosimetry and direct probe measurements The Siemens cadmium sulfide probe was calibrated in air against ^{60}Co and against ^{137}Cs sources of known strength so that the meter reading could be converted into actual dose rate readings in rad/h The entire probe measurement system was then recalibrated in water medium such that characteristics of the system could be directly assessed under conditions more nearly resembling the patient monitoring system The probe exhibits a linear response in air or water This method will be reported in detail elsewhere (WREDE et coll)

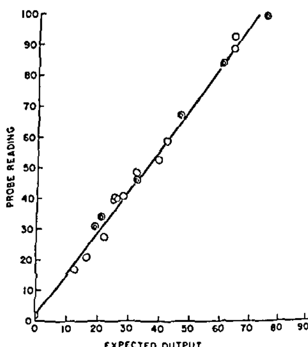


Fig. 2. Dose response curve for probe reading with expected dose rate (rad/h) as calculated by the computer (raw data).

Probe measurements, the relevant expected dose rate in rad/h, and the computer dosimetry calculation for the probe position were correlated (Fig. 2). A comparison of the computer calculated and direct probe measurements are indicated in the figure. These show a close correlation ($r = 0.988$) between the probe reading and the expected computer determined dose rate in the patients forming the material basis for this report. The sensitive detection volume in the probe was situated about mid-way in the probe region for localization and correlation. There was a scatter of the data points about the line indicating that under clinical conditions some probe movement occurs.

Patient experience. An ultimate test of the system is in actual patient implant situations where a complication can be correlated with a high dosage to that structure. The following example illustrates those correlations.

A 33 year old female with a 6 months history of post coital bleeding was found to have an ulcerative lesion of the cervix. Admission and evaluation in June 1971 revealed a Stage IIB carcinoma of the cervix. The patient was treated with 5 000 rad in 5 weeks of whole pelvis radiation using a 4 field technique. A.p. and p.a. fields measured 16 cm × 16 cm in size and lateral fields 8 cm × 15 cm in size. She was admitted for a ^{137}Cs implant in August and 53 mg Ra equivalents inserted for 66 h. or 3 500 milligram hours radium. The left pelvic sidewall received 600 rad of supplemental dose following the implant. Tumor regression was complete. However, 6 months later the patient developed nausea, vomiting

and abdominal distension She was admitted with evidence of a pelvic mass, bowel perforation and sepsis Exploration revealed a pelvic abscess with a rectal perforation posterior to the cervix Retrospective analysis of dose showed that it was 3 155 rad at point A, and 8 000 rad at the rectum at the site of injury (combination of 5 000 rad from external beam therapy and 3 000 rad from the intracavitary implant) (2 6 mCi ^{137}Cs is assumed to be equivalent to 1 mg of radium HORSLER et coll Caesium sources for use in intracavitary and interstitial radiotherapy Brit J Radiol 37 (1964), 385)

Discussion

A simple, accurate and reliable method for the monitoring of radiation dose to all points in the vicinity of the implant is desirable Two systems of dosimetry have been developed One of these depends on routine direct measurements of the dose to the rectum and bladder to determine limiting doses (LEDERMAN 1950, FLETCHER et coll 1958, KOTTMEIER & GRAY 1961) The other depends on computerized dosimetry wherein once the geometry of the implanted source is specified, the computer will rapidly compute dose in three dimensions about the sources and at specified points along structures adjacent to the implant (STOVALL & SHALEK 1962, WEIGENBERT et coll 1967, DURRANCE & FLETCHER 1968) It is apparent that these two systems are mutually supportive and should give the identical analysis of dose provided the same points are assessed A variety of probes and detectors have been described (SHALEK & COLE 1958, MILLION et coll 1966, PECKHAM et coll 1969, SHELDON et coll 1969)

At the same time there is a considerable literature reporting that no correlation of dose and complications in the pelvis was noted where direct measurements have been made in the rectum and bladder (LEDERMAN 1950, DEELEY 1954, STRICKLAND 1954, FLETCHER et coll 1958, PECKHAM et coll 1969) FLETCHER et coll state that 'there is no evidence of absolute levels for the radiation tolerance of the bladder and rectum' They also note the importance of associated factors such as ethnic background, age, associated disease and anomalies. NATHANSON et coll (1967) have also reported poor correlation between calculated and measured dosage PECKHAM et coll (1969) and BUCHLER et coll (1971) have also studied radiation dosage and complications in cervical carcinoma therapy They used direct measurement dosimetry with a Townsend probe and separate computer analysis, each of these techniques being [illegible]

[illegible] allowed to exceed 7 000 rad to the bladder or rectum in early tumor and 8 000 rad in extensive tumors Rectovaginal and vesicovaginal fistulae occurred in only 1 4 per cent but other serious bowel and uretal com

plications were seen in 6 4 per cent Their patients were treated to an average paracervical triangle dose of 10 463 milligram hours radium On inspection of their data it is clear that no rectal injuries were observed at less than 6 000 rad, and 12 per cent injuries with doses in between 7 500 rad and 8 500 rad Nearly all patients receiving over 8 500 rad developed complications However, they concluded that no individual correlation between dose delivered and complications could be demonstrated

KOTTMEIER & GRAY (1961) found that there was a direct correlation with dosage levels in bladder and rectum and injury rate Using Radiumhemmet techniques, no serious complications were observed with less than 4 000 rad as measured by a Sievert ionization chamber They *considered grade I injuries* insignificant When the dose was over 5 000 R by radium application 21 per cent grade II and III injuries was observed in the rectum The bladder tolerated more dose but the injury rate was high where larger doses than 6 000 or 9 000 R were delivered from radium or external irradiation

The latter two reports indicate that injuries can be correlated with total dose to the rectum and bladder However, for an individual patient the likelihood of injury is a complex probability function with many complex variables such as age, associated diseases, ethnic background, etc contributing

Our basic hypothesis has been that complications and high dosage are correlated although not necessarily in any absolute manner Thus as the dosage to a radiation sensitive structure increases, the probability of a complication occurring also rises Naturally there are problems that occur with the unusually sensitive patient, in the presence of prior surgery or infection, or when later surgery is attempted Indeed, VAN NAGELL et coll (to be reported elsewhere) have identified a patient phenotypic profile of unusual sensitivity in the Kentucky population Our basic approach has been that if an adequate tumor dose can be delivered to the tumor region relative to the tolerance of the surrounding normal structure, the probability of tumor control can be maximized and the complication probability minimized Therefore, the basic problem is optimization, and the present report describes the system we have developed for computer dosimetry with a simple and accurate method of direct monitoring of the computer output by a single probe reading The probe in turn guarantees that no error in input of data was made for the computer dosimetry or in loading of implants The system as evolved has permitted us greater confidence in the validity of the system and in delivering accurate patient dosage The two interrelating systems represent a logical extension and interaction of the independent direct probe measurement and computer dosimetry systems We propose to utilize this system in a prospective investigation of radiation dose and complications in gynecologic tumor radiation therapy

SUMMARY

A system has been developed wherein a direct means of monitoring computer dosimetry was investigated. There was linear agreement between probe measurements and expected dose rate calculated by a computer system (correlation coefficient r = 0.988). These two systems were mutually supportive in determining the correctness of loading and in assessing precision of dose near the implanted sources. This system offers a technique for more rigorous analysis of clinical experience. This dual dosimetry system provides a means for greater confidence in implant dosimetry by directly monitoring computer output in gynecologic implant therapy.

ZUSAMMENFASSUNG

Es wurde ein System entwickelt, mit welchem ein direktes Verfahren zur Überwachung der Komputer Dosimetrie untersucht wurde. Es bestand eine lineare Übereinstimmung zwischen den Untersuchungsmessungen und den erwarteten Dosiswerten, die mit Hilfe des Komputer Systems errechnet wurden (Korrelationskoeffizient r = 0.988). Diese beiden Systeme stützten einander bei der Bestimmung der Richtigkeit der Ladung und bei der Feststellung der Genauigkeit der Dosis in der Nähe der implantierten Strahlenquellen. Dieses System stellt eine Technik zur besseren Analyse der klinischen Erfahrung dar. Das zweifache Dosimetrie System bildet ein Verfahren für eine grössere Sicherheit bei der Dosimetrie durch direkte Komputer Überwachung bei der gynäkologischen Implantationstherapie.

RÉSUMÉ

Les auteurs ont mis au point un système qui a permis d'étudier un moyen direct de représentation sur un écran de la dosimétrie sur ordinateur. Il y a une concordance linéaire entre les mesures par sonde et le débit de dose prévu calculé aussi par un système d'ordinateur (coefficient de corrélation r=0.988). Ces deux systèmes contribuent à déterminer la charge correcte et à évaluer la précision de la dose près des sources implantées. Ce système permet une analyse plus rigoureuse de l'expérience clinique. Ce double système de dosimétrie permet d'avoir une plus grande confiance dans la dosimétrie d'implantation en montrant directement sur un écran les résultats de l'ordinateur dans le traitement par implantation en gynécologie.

REFERENCES

BUCHLER, D. A., KLINE, J. C., PECKHAM, B. M., BOONE, M. L. M. and CARR, W. F.: Radiation reactions in cervical cancer therapy. Amer. J. Obstet. Gynec. 111 (1971), 745.

DEELEY, T. J.: Routine measurements of rectal dose in treatment of carcinoma of cervix by radium. J. Fac. Radiol. 5 (1954), 289.

DURRANCE, F. Y. and FLETCHER, G. H.: Computer calculations of dose contribution to regional lymphatics from gynecological radium insertions. Radiology 91 (1968), 140.

FLETCHER, G. H., BROWN, T. C. and RUTLEDGE, F. N.: Clinical significance of rectal and bladder dose measurements in radium therapy of cancer of the uterine cervix. Amer. J. Roentgenol. 79 (1958), 421.

WALL, J. A., BLOEDORN, F. G., SHALEK, A. J. and WOOTON, R.: Direct measurements and isodose calculations in radium therapy of carcinoma of the cervix. Radiology 61 (1953), 885.

KOTTMEIER H L and GRAY M L Rectal and bladder injuries in relation to radiation dose in carcinoma of the cervix Amer J Obstet Gynec 82 (1961), 74

LEDERMAN M *Some clinical applications in radium therapy of a probe type direct reading* dosage rate meter Brit J Radiol 23 (1950), 430

MILLION R R, MAUDERLI W and BRUNO F D Modifications of technic for bladder and rectal measurements in carcinoma of the cervix Radiology 86 (1966), 921

NATHANSON B N, LIEGNER L M, RUDOLPH J D and HODARA M Dose verification studies in radium therapy of carcinoma of the cervix Amer J Obstet Gynec 97 (1967) 808

PECKHAM B M, KLINE J C, SCHULTZ A F, CAMERON J R and VERMUND F Radiation dosage and complications in cervical cancer therapy Amer J Obstet Gynec 104 (1969), 485

SHALEK R J and COLE A A scintillation probe for the measurement of radiation dosage in body cavities Amer J Roentgenol 79 (1958), 450

SHELDON J J, VUKSANOVIC M, WOLD G J and JONES F A A new rectal dosimetry catheter for use in radiation therapy of carcinoma of the uterine cervix Amer J Roentgenol 105 (1969) 120

SIEVERT R M Eine Methode zur Messung von Röntgen, Radium und Ultrastrahlungen nebst einigen Untersuchungen uber die Anwendbarkeit derselben in der Physik und der *Medizin Acta radiol (1932) Suppl No 14*

STOVALL M and SHALEK R J Study of explicit distribution of radiation in interstitial implantations I Method of calculation with automatic digital computer Radiology 78 (1962) 950

STRICKLAND R Damage in rectum in radium treatment of carcinoma of cervix Brit J Radiol 27 (1954) 630

WEIGENBERT I J, MARSHALL J R and POWERS W E Radiolucent intracavitary radiation applications Their special usefulness in computer calculated dosimetry Amer J Roentgenol 99 (1967) 881

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SIALIC ACID AND NEURAMINIDASE AFTER WHOLE BODY IRRADIATION OF RATS

D KOCMIERSKA GRODZKA, G B GERBER and J-P DECOCK

Sialic acids are constituents of many glycoproteins as well as of gangliosides in the brain (GINSBURG & NEUFELD 1969) They have been implicated recently in many important functions such as maintaining the intercellular gap (GENT et coll 1966, LANGLEY 1971), regulating the catabolism of glycoproteins (VAN DEN HAMER et coll 1970, MORELL et coll 1971) and influencing the state and charge of lysosomal enzymes (HIGASHINO et coll 1972, GOLDSTONE et coll 1971) For these reasons it was thought of interest to investigate whether sialic acid in organs and body fluids as well as neuraminidase are affected by whole body irradiation

Methods Male rats of the Wistar strain, weighing about 250 g, were exposed to whole body irradiation (250 kVp, FSD 57 cm 1 4 mm Cu HVL 100 R/min) The irradiated animals as well as the controls were starved, but had access to water, some were maintained in metabolism cages for the collection of urine
One or [illegible]
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Submitted for publication 5 February 1973

KOTTMEIER H L and GRAY M L Rectal and bladder injuries in relation to radiation dose in carcinoma of the cervix Amer J Obstet Gynec 82 (1961), 74

LEDERMAN M Some clinical applications in radium therapy of a probe type direct reading dosage rate meter Brit J Radiol 23 (1950), 430

MILLION R R, MAUDERLI W and BRUNO F D Modifications of technic for bladder and rectal measurements in carcinoma of the cervix Radiology 86 (1966), 921

NATHANSON B N, LIFENER L M, RUDOLPH J D and HODARA M Dose verification studies in radium therapy of carcinoma of the cervix Amer J Obstet Gynec 97 (1967) 808

PECKHAM B M, KLINE J C, SCHULTZ A E, CAMERON J R and VERMUND F Radiation dosage and complications in cervical cancer therapy Amer J Obstet Gynec 104 (1969), 485

SHALEK R J and COLE A A scintillation probe for the measurement of radiation dosage in body cavities *Amer J Roentgenol 79 (1958), 450*

SHELDON J J, VUKSANOVIC M, WOLD G J and FONTS F A A new rectal dosimetry catheter for use in radiation therapy of carcioma of the uterine cervix Amer J Roentgenol 105 (1969) 120

SIEVERT R M Eine Methode zur Messung von Röntgen Radium und Ultrastrahlungen nebst einigen Untersuchungen uber die Anwendbarkeit derselben in der Physik und der Medizin Acta radiol (1932) Suppl No 14

STOVALL M and SHALEK R J Study of explicit distribution of radiation in interstitial implantations I Method of calculation with automatic digital computer Radiology 78 *(1962), 950*

STRICKLAND R Damage in rectum in radium treatment of carcinoma of cervix Brit J Radiol 27 (1954) 630

WEIGENBERT I J, MARSHALL J R and POWERS W E Radiolucent intracavitary radiation applications Their special usefulness in computer calculated dosimetry Amer J Roentgenol 99 (1967), 881

Table 2

Sialic acids in rat serum after whole body irradiation

Dose (R)	Sialic acids nmole/100 ml serum ± SE	
	1 day	*2 days*
0	279±9	297±7
(starved)	(4.38±1.01)	(4.57±0.34)
500	344±22*	324±8**
	(4.96±0.25)	(4.75±0.21)
2 000	364±9*	283±7
	(4.92±0.21)	(6.18±0.27)*

Values in parenthesis are free sialic acids Values of fed controls 279±9 (4.60±0.10) are not significantly different from starved controls, 10—14 animals were used per experimental point

Difference statistically significant (* $p < 0.01$, ** $p < 0.05$)

neuraminidase) The sialic acids were determined by the thiobarbituric acid reaction of WARREN (1959) with 50 μl homogenate serum or urine for the assay (200 μl for free sialic acid in serum) and 2 ml cyclohexanone for extraction Free sialic acids were determined directly in the homogenates and total sialic acids after hydrolysis in 0.1 N H_2SO_4 at 80° for one hour The calculations were carried out on the basis of measurements of O D at 532 and 562 nm Protein was determined by the Biuret reaction and creatinine in urine with alkaline picrate Neuraminidase (N-acetylneuraminate glycohydrolase, E C 3.2.1.18) was measured in the supernatant of the 25 per cent (spleen 12.5 %) homogenate after centrifugation for 105 000 g × h The assay was carried out with 0.2 ml supernatant and 50 μg neuramin lactose by a slight modification of the method of CARUBELLI & TULSIANI (1971) The value of free sialic acid in supernatant was subtracted from the value obtained with substrate after incubation

Results

Data on the content of sialic acids in organs presented in Table 1 reveal that liver contains much less sialic acids than the other organs investigated Part of this material might have been associated with serum glycoproteins Experiments were therefore carried out also with liver washed free from blood

Table 1

Sialic acid content in organs of whole body irradiated rats. Sialic acid (µmol/g tissue) ± SE

Dose (R)	Liver 24 h	Liver 48 h	Liver washed 24 h	Liver washed 48 h
0	0.821 ± 0.041	0.718 ± 0.082	0.681 ± 0.062	0.512 ± 0.085**
(starved)	(0.199 ± 0.012)	(0.247 ± 0.019)	(0.195 ± 0.012)	(0.187 ± 0.012)**
0	0.787 ± 0.035			
(fed)	(0.224 ± 0.007)			
500				
2 000	0.643 ± 0.020****	0.623 ± 0.024	0.460 ± 0.051****	0.580 ± 0.015
	*(0.215 ± 0.013)****	*(0.291 ± 0.027)*	*(0.202 ± 0.012)*	*(0.315 ± 0.019)*****

Dose (R)	Brain 24 h	Brain 48 h	Spleen 24 h	Spleen 48 h
0	3.48 ± 0.26	3.48 ± 0.29	5.89 ± 0.181	6.32 ± 0.32
(starved)	(0.450 ± 0.017)	(0.430 ± 0.017)	(0.840 ± 0.056)	(0.831 ± 0.040)
0	3.43 ± 0.22		4.52 ± 0.22	
(fed)	(0.505 ± 0.016)		(0.635 ± 0.016)**	
500	3.24 ± 0.40	3.34 ± 0.16	6.83 ± 0.33***	8.35 ± 0.54****
	(0.439 ± 0.031)	(0.348 ± 0.011)	(1.06 ± 0.071)***	(0.918 ± 0.23)
2 000	3.30 ± 0.23	2.51 ± 0.20***	7.88 ± 0.23****	6.85 ± 0.23
	(0.451 ± 0.017)	(0.351 ± 0.011)****	(1.198 ± 0.011)****	(1.086 ± 0.053)****

Dose (R)	Lung 24 h	Lung 48 h
0	2.19 ± 0.11	1.79 ± 0.17*
(starved)	(0.485 ± 0.038)	(0.414 ± 0.027)
0	*3.07 ± 0.37*	
(fed)	(0.346 ± 0.021)	
500	2.13 ± 0.10	4.16 ± 0.75***
	(0.572 ± 0.030)	(0.495 ± 0.025)
2 000	2.53 ± 0.26	2.79 ± 0.07****
	(0.631 ± 0.033)****	(0.606 ± 0.057)****

Values in parenthesis are free, the others total sialic acid
Values significantly different after starvation (* $p < 0.05$, ** $p < 0.01$) and after irradiation compared to starved controls (*** $p < 0.05$, **** $p < 0.01$)

Table 4

Soluble neuraminidase activity in tissues of irradiated rat

Dose (R)	Activity nmole/h/g tissue					
	Liver		Brain		Spleen	
	24 h	48 h	24 h	48 h	24 h	48 h
0	29.6±2.6	30.4 ± 9.2	35.7±3.6	27.0±4.3	37.6±5.0	59.7±9.8
	(64.5±3.2)	(70.2 ± 1.6)	(59.8±4.2)	(70.5±7.2)	(126 ± 8)	(141 ±13)
500	21.7±9**	34.5 ± 4.0	16.1±2.6	36.1±3.3	27.4±6.2*	43.5±5.8
	(91.7±2.8)**	(97.4 ± 2.5)**	(55.3±1.5)	(48.3±2.8)*	(152±7)**	(163 ±7)
2000	17.8±4.1*	20.53± 6.2	no significant activity**	22.5±5.6	no significant activity**	45.4±15
	(70.1±6.2)	(90.3 ±17.2)	(58.7±7.5)	(53.6±3.7)	(175 ±10)**	(133 ±14)

Only activity/g tissue is given. The protein concentration in the supernatant was: liver 50 mg/g, brain 12.4 mg/g, spleen 26.8 mg/g (changes in protein content after irradiation are small and do not significantly alter the results).
Values in parentheses are free sialic acid in supernatant (nmole/g) without incubation with neuraminyl lactose. Values significantly different (*$p < 0.05$ **$p < 0.01$)

Neuraminidase activity in the liver, brain and spleen diminishes after both 500 R and 2000 R (Table 4). *Conversely, free sialic* acid is increased in the liver one and two days after 500 R (but not after 2000 R) and in the spleen one day after these doses.

Discussion

The most important observations to be discussed are: a *decrease in the total* sialic acid in the liver, an increase in the spleen, lungs, serum and urine, and a decrease in the neuraminidase activity in the liver, brain and *spleen. Three* aspects of the function of sialic acid which could become important in the radiation syndrome must thus be considered. Sialic acid is an important *constituent* of the cell membrane and is thought to maintain the gap of about 250 Å between cells (Gent et coll., Langley).

Cells may, in the absence of sialic acid, approach one another to under 50 Å. Changes in the surface properties of cells have among others been implicated *in* the mechanism of malignancy and in immune and phagocytic reactions. Changes in the surface structure of cell membranes after irradiation have also been reported for *erythrocytes*, cancer cells and others (Gerber & Altman 1971).

Table 3

Sialic acid in rat urine after whole body irradiation

Dose (R)	Sialic acid content (nmole/μmole creatinine) ± SE		
	0–24 h	24–48 h	48–72 h
0	2.06±0.07	2.01±0.13	2.01±0.24
(starved)	(2.44±0.215)	(2.26±0.51)	
300	3.22±0.10*	2.93±0.17*	2.43±0.31
500	3.10±0.15*	2.17±0.19	
	(4.49±0.34)*	(1.82±0.19)	
700	3.95±0.34*	3.02±0.25*	2.49±0.29
1 000	3.79±0.41*	2.89±0.24*	2.77±0.14
1 500	4.90±0.33*	3.06±0.12*	4.73±0.26*
2 000	4.49±0.77*	2.23±0.17	
	(4.20±0.16)*	(2.23±0.19)	

The data are derived from two experiments with 12 animals per point. The first (500 and 2 000 R) set of excretion values appeared to return more rapidly to normal. Data in parenthesis are total sialic acid, the others free sialic acid. Differences statistically significant (*$p < 0.01$).

This treatment lowered significantly its sialic acid content but changes after irradiation occurred to about the same extent. Total liver sialic acid tends to decrease during starvation, the total liver sialic acid diminishes significantly on the first day after irradiation whereas the free sialic acid increases slightly. A decrease is evident in the brain on the second day after high doses, the lungs display a decrease in total sialic acid and a rise in the free acid during starvation with an increase in both parameters after irradiation. Total and free sialic acid in the spleen increases when the data are related to g tissue or g protein. Due to the fall in organ weight, sialic acid in the total spleen diminishes however to less than 50 per cent of the controls. The increase in sialic acid in lymphoid organs was followed in more detail after doses of from 100 to 800 R and time periods of from 1 to 5 hours. These data were not recorded since only a slight increase was observed 3 and 5 hours after high doses. The changes in sialic acid thus do not take place before the organ weight diminishes. The total sialic acid in rat serum increases significantly on days 1 and 2 whereas free sialic acid is enhanced only 2 days after 2 000 R when the levels of total sialic acid have again declined (Table 2).

The excretion into the urine of free (and total) sialic acid is elevated significantly after doses of 300 R and more, a dependency from the radiation dose exists with saturation after high doses (Table 3).

radiation injury since it is easy to execute and responds readily to low doses of radiation On the other hand, the differences in urinary excretion of sialic acid at various dose levels are not marked and saturation seems to occur after high doses Moreover, the test is non-specific and certain pathologic conditions, such as myocardial infarction (MAURY & HUTTUNEN 1972), chronic bronchitis (ATASSI et coll 1959) are claimed to give rise to an increase in the urinary sialic acid Preliminary experiments in irradiated primates have indicated that excess excretion of sialic acid is not confined to rats alone

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SUMMARY

Total and free sialic acid as well as soluble neuraminidase were determined *in the liver*, brain and spleen of whole body irradiated rats The total sialic acid in the liver proved to decrease and that in the spleen and brain to increase whereas the soluble neuraminidase in most organs diminished The sialic acids in the serum and urine increased so that their ex cretion might be a reaction suitable for detecting radiation injury

ZUSAMMENFASSUNG

Gesamte und freie Neuraminsauren, sowie losliche Neuraminidase wurden in Leber Gehirn und Milz ganzkorperbestrahlter Ratten bestimmt Die gesamte Neuraminsaure in Leber nimmt ab die in Milz und Gehirn nimmt zu, wahrend losliche Neuraminidase gewohnlich vermindert ist Neuraminsauren in Serum und Urin sind *vermehrt und ihre* Ausscheidung konnte eventuell zu Erkennung des Strahlenschadens dienen

RÉSUMÉ

Les auteurs ont determine l acide sialique totale et libre et la neuraminidase soluble dans le foie *le cerveau et la rate de* rats ayant subi une irradiation corporelle totale L aride sialique total dans le foie decroit, il augmente dans la *rate et dans le cerveau* alors que la neuraminidase soluble diminue dans la plupart des organes Les acides sialiques dans le serum et l urine *augmentent il* en resulte que leur excretion pourrait etre une reaction convenant pour detecter les dommages dus a l irradiation

REFERENCES

ATASSI M Z BARKER S A HOUGHTON L E and STACEY M Neuraminic acid and its relation to chronic bronchitis Clin chim Acta 4 (1959) 741

CARUBELLI R and TULSIANI D R P Neuraminidase activity in brain and liver of rats during development Biochim biophys Acta 237, (1971), 78

and it has been claimed that the earliest damage detectable in irradiated lymphocytes is represented by the loss of the surface coat (WOŹNIEWICZ 1972) The present data on total lymphoid tissues suggest an increase in concentration of sialic acid rather than a decrease although this may be the result of the loss of cellular components with a low sialic acid content Research on isolated lymphocytes must eventually clarify whether injury to sialic acid in the cell membrane is indeed an early expression of radiation injury

The metabolism of serum glycoproteins is determined by their sialic acid content and that of the liver cell membrane Glycoproteins from which sialic acid has been removed are readily absorbed by liver cell membranes (MORELL et coll 1971, VAN DEN HAMER et coll 1970) and may thus become disposed of by catabolism and lack of sialic acid in liver cell membranes (PRICER & ASHWELL 1971) depresses the binding of glycoproteins Indeed, serum glycoproteins have been reported to increase in response to lethal irradiation (EVANS et coll 1968), adequately confirmed by the present findings of an increase in sialic acid bound in serum Moreover, it would appear that certain types of serum glycoproteins, the haptoglobins, increase significantly as early as 5 hours after irradiation (KOCMIERSKA-GRODZKA & GERBER 1973) Synthesis of $\alpha 2$-serum globulins is apparently not greatly altered after irradiation (SASSEN et coll 1965, GERBER & ALTMAN), it is thus possible that decreased catabolism regulated by the sialic acid of liver membranes is responsible for the changes present in the serum

One final important aspect in the function of sialic acid and neuraminidase is related to the activity of lysosomal enzymes Hydrolytic enzymes in lysosomes were reported to exist in an acid and basic form (HIGASHINO et coll, GOLDSTONE et coll), the latter being formed by removal of sialic acids from the glycoprotein enzymes The acid form is thought not only to have different physicochemical properties but also to be more susceptible to be released into the cytoplasma It is well known that after irradiation lysosomal enzymes are present in a more labile state (GERBER & ALTMAN) and it is conceivable that this is related to a decrease in neuraminidase in relation to sialic transferase Neuraminidase could thus be a factor regulating and promoting hydrolytic breakdown in tissue after irradiation Further investigations with electrophoresis and assay of the transferase of lysosomal enzymes must decide whether this is possible, and at which points sialic acid may intervene in the auto and heterolytic reactions after irradiation

The increased excretion of sialic acid involves mainly free sialic acid This material may be derived from sialic acid degraded in radiation sensitive organs or may represent catabolic products from excess glycoproteins excreted Nevertheless, this test might eventually become useful as a biochemical indicator of

FROM THE DEPARTMENT OF GYNECOLOGIC RADIATION THERAPY (DIRECTOR PROF O KJELLGREN), UMEÅ UNIVERSITY, 901 85 UMEÅ, SWEDEN

GASTROINTESTINAL PROTEIN LOSS INDUCED BY ^{60}CO IRRADIATION OF THE ABDOMEN IN MICE

B FRANKENDAL

The treatment of ovarian carcinoma comprises surgery, irradiation and chemotherapy with irradiation playing an essential role in certain types of the condition

When only orthovoltage roentgen radiation with energies of 170 to 250 kV were formerly available, the delivery of a satisfactory dose to regions of possible spread in the abdomen and pelvis was difficult The largest permissible dose was limited by radiation reactions of the skin as well as by the unfavourable distribution of the absorbed dose between soft and bony tissues Modern super voltage roentgen machines and isotope teletherapy units have now, however, made it possible to apply larger tumour doses to the abdomen and pelvis without these limitations. This has, however, created new problems and raised new questions Irradiation of ovarian carcinoma must often be extended to large areas of the abdomen and pelvis High energy radiation causes radiation reactions of the gastrointestinal tract so that these as well as the radiation sensitivity of the kidneys now constitute the limiting factors

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EVANS A S, QUINN F A, BROWN J A and STRIKE T A Effect of ionizing radiation on total protein bound neutral hexoses in the plasma of mice Radiat Res 36 (1968) 128
GENT W L G, ROUNCE J R and WILLIAMS P L The electron microscopy of red cell membranes contacts J Anat 100 (1966), 447
GERBER G B and ALTMAN K I Radiation Biochemistry Vol II Edited by K I Altman G B Gerber and S Okada Academic Press New York 1971
GINSBURG V and NEUFELD E F Complex heterosaccharides of animals Ann Rev Biochem 38 (1969), 371
GOLDSTONE A, KONECNY P and KOENIG H Lysosomal hydrolases Conversion of acidic to basic corms by neuraminidase FEBS Letters 13 (1971), 68
HIGASHINO K, HASHINOTSUME M and YAMAMURA K Effect of neuraminic acid removal on the properties of a variant alkaline phosphatase in hepatoma Clin chim Acta 40 (1972) 305
KOCMIERSKA GRODZKA D and GERBER G B Haptoglobin levels after irradiation of rats In press
LANGLEY O K Sialic acid and membrane contact relationship in peripheral nerve Exp cell Res 68 (1971), 97
MAURY P and HUTTUNEN J K Effect of age sex pasting and surgery on the excretion of neuraminyl lactose and neuraminyl N acetyl lactosamine in rat urine Clin chim Acta 37 (1972), 433
MORELL A G, GREGORIADIS G, SCHEINBERG I H HICKMAN J and ASHWELL G The role of sialic acid in determining the survival of glycoproteins in the circulation J biol Chem 246 (1971), 1461
PRICER JR W E and ASHWELL G The binding of desialylated glycoproteins by plasma membranes of rat liver J biol Chem 246 (1971), 4825
SASSEN A GERBER G B, KENNES F and REMY DEFRAIGNE J Serum protein synthesis after irradiation Radiat Rest 25 (1965), 158
TULSIANI D R P and CARUBELLI R Studies on the soluble and lysosomal neuraminidase of rat liver J biol Chem 245 (1970) 1821
VAN DEN HAMER C J A MORELL A G, SCHEINBERG I H HICKMAN J and ASHWELL G Physical and chemical studies on coruloplasmin J biol Chem 245 (1970) 4397
WARREN L The thiobarbituric acid assay of sialic acids J biol Chem 234 (1959) 197
WOZNIEWICZ B A Surface coat of thymocytes in radiation research IXth Annual Meeting European Society of Radiation Biology Rome 1972

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Irradiation of the abdomen may produce such symptoms as nausea, vomiting anorexia, colicky pain, tenesmus, diarrhoea and loss of body weight, these are due mainly to radiation-induced gastrointestinal changes (CONARD 1956)

Proliferating cells of the crypts in the mucosa of the small intestine are very sensitive to ionising radiation so that this mucosa is one of the most radiation sensitive tissues of the body Irradiation with a single dose of about 100 rad is sufficient to inhibit mitotic activity in the cells of the crypts within an hour (WILLIAMS et coll 1958) The radiation reactions of these cells following whole body *irradiation with sublethal doses in mice soon disappear* (HORNSEY & VATISTAS 1963, WIERNIK et coll 1966) Whole body irradiation with larger doses completely inhibits mitotic activity in the crypt cells so that no new cells can migrate up to the tips of the villi This gradually results in denudation of the villi (BOND 1963)

QUASTLER (1956) stated that a causal relationship exists between the histologic changes in the epithelium of the small intestine and the death of experimental animals after irradiation The animals survived for 3 to 5 days, the duration of their survival not varying with the size of the radiation dose above a certain threshold level, the effect was the same whether the abdomen alone or the whole body was irradiated The deaths that occurred within five days were therefore ascribed to acute gastrointestinal radiation reactions BOND (1963) *considered that loss of fluid and electrolytes from the bloodstream into the* small intestine after radiation was probably due to denudation of the mucosa of the small intestine This explanation was however, questioned by LUSHBAUG (1969), who felt that the cause of the radiation-induced losses via the gastrointestinal tract should be sought in disorders of the vascular system of the small intestine (EDDY & CASARETT 1968) That radiation has an undesired effect on the circulation of the blood in the intestine has been demonstrated by JANOSSY (1969) KABAL et coll (1972) surmised that this effect on the circulation is probably due to a local radiation induced alteration of the neurohumoral vascular regulation

Irradiation of the abdomen apparently produces gastrointestinal protein loss in experimental animals (WETTERFORS et coll 1965, BROMFIELD & DYKES 1964, VATISTAS & HORNSEY 1966, FRIEDBERG et coll 1961, HORNSEY & VATISTAS 1968) SULLIVAN (1961) reported that the loss of albumin in irradiated animals was due to leakage mainly via the jejunum Irradiation of the abdomen also induces gastrointestinal protein loss in man (*BIRKE et coll 1967*, LOBE & SCHNEIDER 1967) DALLA PALMA et coll (1963) discovered no increase in the leakage of polyvinylpyrrolidone in patients who had received external irradiation of the abdomen and pelvis with a telecobalt technique in a total dose of 5 000 rad to the abdomen over about 25 treatments



Fig 1 Plastic case placed on the lead plate with a rectangular window through which the abdomen of the mouse is irradiated from below

Several methods have been employed in the investigation of gastrointestinal protein loss after irradiation of the abdomen Most of these have been used with high molecular weight substances, such as polyvinylpyrrolidone, dextran or protein labelled with radioactive isotopes and administered intravenously, the labels most common are ^{131}I and ^{51}Cr

GRAY & STERLING (1950) demonstrated that $^{51}CrCl_3$ given intravenously united with plasma proteins and opened up a new and simple approach to the investigation of leakage of high molecular weight substances from the bloodstream to the intestine FRANKENDAL & STIGBRAND (1973) have revealed that 10 μCi $^{51}CrCl_3$ given intravenously to mice will demonstrate about 70 per cent of all protein bound tracer in the transferrin fraction in two hours At 24 hours after the injection the corresponding figure is more than 90 per cent This protein has a molecular weight of about 90 000

EBAUGH et coll (1958) indicated that if erythrocytes be labelled with sodium radiochromate and given by mouth, the label will be almost completely excreted with the faeces and that only a minor portion will be absorbed WALDMANN et coll (1969) obtained similar results with ^{51}Cr albumin ROOTWELT (1966) compared ^{131}I PVP (polyvinylpyrrolidone), ^{131}I albumin and $^{51}CrCl_3$ as tracers in the investigation of gastrointestinal protein loss, they reported that the last mentioned radioisotope was the most suitable one

Since the gastrointestinal reaction is of great importance as a limiting factor in the irradiation of the abdomen and since the reaction leads to protein leakage, it was considered worthwhile to determine certain aspects of these problems The purpose of the present investigation was therefore to ascertain the extent to which the gastrointestinal protein loss varies with the size of the dose as well as with the interval after irradiation

Material and Methods Mice were considered suitable for the investigation, first, because they do not react with diarrhoea to doses up to a sublethal level,

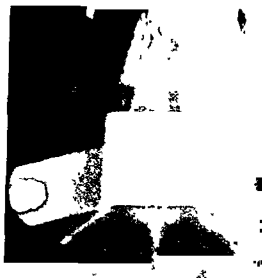


Fig. 2. Roentgenogram of mouse held in the plastic case. The rectangular field in the middle covers the irradiated part of the animal.

this is important, and leaves the separate collection of the faeces and urine uncomplicated. Secondly because, like human beings, mice are omnivorous.

The effect of different doses of radiation on gastrointestinal protein loss, as judged from the activity of the faeces, was investigated in a series of 98 female mice of NMRI strain, aged 70 to 100 days and weighing 27 to 30 gram, the animals had free access to water and food (commercial pellet diet). They were placed in a plastic case to limit their mobility, and the abdomen was irradiated with a telecobalt unit, the case made the experimental conditions reproducible (Fig. 1). The animals were lightly anaesthetised with ether before being placed in the frames; this was necessary so that the positions could be reproduced, the mice were not under an anaesthetic during irradiation. The case containing a mouse was afterwards placed on a 4.5 cm thick lead plate. This plate had a rectangular window (3 cm × 3.5 cm) corresponding to the abdominal area of the animal, and was put on the cobalt machine so that the abdomen could be irradiated from below (Fig. 2). The source to skin distance was 35 cm and the absorbed dose rate 860 ± 10 rad/min ($\overline{X} \pm 1$ SD), as calculated from determination of the exposure rate with a Siemens thimble ionization chamber. The homogeneity of the field was checked with small ionization chambers in the plastic case.

The range of variation in the field was less than 2 per cent, the dose being

determined at a point corresponding to 3 mm below the surface of the abdomen The dose through the animals' abdomen was practically constant, the sagittal diameter of the animal when in the case never exceeded 1 4 cm The animals were irradiated with a total dose of 500, 1 000, 2 000 or 3 000 rad given in daily fractions of 500 rad

The controls were treated in exactly the same way as the experimental animals except that they were not irradiated, they were contained in plastic cases for the same time as the experimental animals Each experimental group as well as each control group consisted of about 20 animals

Three days after the last irradiation 10 μCi $^{51}CrCl_3$ diluted in isotonic saline to 0 4 ml was injected into a tail vein of all the experimental and control animals The isotonic solution of $^{51}CrCl_3$ (Amersham, England) had a specific activity of 100 to 300 μCi/μg chromium, the amount of chromium injected into each animal was less than 0 1 μg An isotonic saline solution was introduced first in order to control that the tip of the needle was situated within a vein The isotope solution was then injected followed by a smaller volume of the isotonic saline The activity in the syringe before and after the injection and that in the irradiated animal were measured with a scintillation counter The mice were put in the plastic case beneath the scintillation counter during the counting procedure so as to secure constant geometry, this made it possible to check that the animals had received the amount of the isotope intended

The animals were afterwards housed in separate metabolic cages with cellulose beneath the wire netting The urine was absorbed by the cellulose while the faeces lay upon it Every 24 hours the faeces were collected and placed in plastic vials and at the same time the cellulose was removed to counting vessels The faeces and urine were collected for four days

Six mice were examined with the same technique and given 10 μCi $^{51}CrCl_3$ intravenously in a preliminary examination The faeces were collected for ten days and their content of ^{51}Cr was measured, more than 85 per cent of the excreted isotope appeared with faeces collected during the first four days It was therefore considered sufficient to limit the collection of the faeces and urine to four days

The faecal activity was measured in a well type scintillation counter Three samples were taken from the injection solution with a *micropipette* (2 μl) and the activity in each pipette was measured four times This method indicated that the determination of a given activity of the isotope varied by less than $\pm$ 1 per cent with a probability of 95 per cent The activity in the faeces collected for four days was then added The same procedure was used for examination of the urine samples The losses of $^{51}CrCl_3$ with the faeces and urine were expressed relative to the activity of the isotope injected

Table 1

^{51}Cr in faeces collected on first four days following intravenous injection of the isotope given three days after the end of irradiation of mice with a varying total dose. The values are expressed as percentages of the dose injected.

Dose (rad)	Faecal excretion (%)		
	n	$\bar{x}$	SD
0	22	4.6	1.6
500	19	5.2	1.3
1 000	20	8.2	1.6
2 000	19	8.0	1.9
3 000	18	9.2	3.1

One factor of a technical nature is of importance in the evaluation of this investigation. ROOTWELT (1966) reported that when 10 to 35 μCi $^{51}CrCl_3$ was given intravenously in 0.5 ml 0.9 % saline to patients about 20 per cent of the activity remained in the syringe after the injection; he therefore aspirated blood and reinjected it so as to empty the syringe as completely as possible. In the present investigation another technique was used: the measurement of the activity in the syringe before and after injection revealed that the desired activity had really been injected and that none remained in the lumen or on the walls of the injection needle. By measuring the activity in the animal under a scintillation detector immediately after injection, it was also possible to correct for small differences in the activity of the tracer injected.

Twenty mice were given a total dose of 1 000 rad (500 rad daily) and observed for 80 days to assess the effect of variation of the radiation dose on survival. A further 18 animals received 3 000 rad in fractions of 500 rad daily and were observed until they died.

The effect of radiation was investigated in a series of 207 female mice at various intervals after the end of the treatment; these animals also received an injection of 10 μCi $^{51}CrCl_3$ intravenously into the tail vein. The injections were given at different intervals after the end of irradiation, 129 of the 207 mice being irradiated with 1 000 rad with the above technique and fractionation; the remaining 78 mice served as controls and were treated in the same way with the exception of irradiation.

The animals were divided into groups. The first group was examined immediately after the end of irradiation. The other groups were examined at 3, 6, 10, 14, 28 and 75 days. Each group consisted of about 20 animals except the one

Table 2

Significance of differences between faecal excretion of ^{51}Cr following different radiation doses

Radiation doses compared (rad)	Significance of difference
0– 500	—
0–1 000	***
0–2 000	***
0–3 000	***
500–1 000	***
500–2 000	**
500–3 000	***
1 000–2 000	
1 000–3 000	—
2 000–3 000	—

examined at 75 days, which was made up of only 10 animals The faeces and urine were collected for four days after the injection of $^{51}CrCl_3$ and the excretion of the isotope was examined by the technique described The total excretion of the tracer in the faeces and urine was expressed as a percentage of the dose

Ten animals were irradiated with 1 000 rad in fractions of 500 rad to ascertain whether irradiation caused gastrointestinal bleeding The faeces were examined every day for heme (Heme-test Ames, England) for 28 days after the irradiation The faeces from all animals were weighed in order to determine whether this represented any difference between the irradiated and unirradiated animals

Statistical methods The results of the measurements are presented with the mean values ± 1 SD Student's t-test and analysis of variance were used in comparison of the means of series to assess whether the differences were significant The levels of signicicance were as follows —= not significant, * = 95 %, ** = 99 %, *** = 99 9 %

The body weights of the mice before and after irradiation were compared with the t test for paired observations

Results

Variation of gastrointestinal protein loss with the radiation dose The faecal excretion, during the first four days, of the radioisotope, $^{51}CrCl_3$, given intravenously to unirradiated mice proved to be 4 6 ± 1 6 per cent (Table 1) Mice irradiated with 500 rad had a somewhat higher mean value, namely

Table 3

^{51}Cr in faeces collected on first four days following intravenous injection of the isotope given at varying intervals after the end of irradiation

Days after irradiation	Faecal excretion (%)						Significance of difference
	Irradiated			Unirradiated			
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	
0	18	5 2	1 4	10	4 6	1 5	–
3	20	8 2	1 6	22	4 6	1 6	***
6	21	6 7	2 0	9	4 6	0 9	**
10	21	5 9	1 5	10	4 3	1 3	**
14	20	6 1	1 4	10	4 2	1 0	***
28	19	5 9	2 2	10	4 3	0 6	*
75	10	6 3	1 6	7	4 6	0 5	*

5 2 ± 1 3 per cent (Table 1) The difference was not significant ($p > 0\ 05$) (Table 2) On the other hand, it is clear from Tables 1 and 2 that the activity of the faeces from the mice irradiated with 1 000 rad was higher (8 2 ± 1 6 per cent, $p < 0\ 001$ and $p < 0\ 001$) than the corresponding values for the two earlier groups, control group and group given 500 rad Any further increase in the dose was not followed by any further significant increase in faecal activity (Tables 1, 2) The values after doses of 2 000 and 3 000 rad (Tables 1, 2) were significantly higher than after 500 The mice irradiated with 2 000 or 3 000 rad appeared to be in a poor general condition during the period covered by the collection of the faeces

Table 4

Weight (g) of faeces collected for four days starting three days after the end of irradiation with a varying total dose

Dose (rad)	Weight of faeces (g)			Significance of difference from 0 rad
	n	$\bar{x}$	SD	
0	22	3 42	1 02	
500	19	3 71	0 85	
1 000	20	3 45	0 73	
2 000	19	3 80	1 00	–
3 000	18	3 88	1 28	–

Table 5

Weight (g) of faeces collected on four consecutive days from unirradiated mice and from those irradiated with 1 000 rad The collection was started at varying intervals after the end of irradiation

Days after irradiation	Weight of faeces (g)						Significance of difference
	Irradiated			Unirradiated			
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	
0	18	3 42	0 61	7	3 80	0 37	–
3	20	3 45	0 73	22	3 42	1 02	–
6	21	3 93	1 13	9	3 87	0 98	–
10	21	3 85	0 60	10	4 00	0 40	–
14	20	3 74	0 76	10	4 10	0 27	–

Variation of gastrointestinal protein loss with the interval after the end of irradiation In those experiments where the collection of the faeces was started immediately after the end of irradiation with a total dose of 1 000 rad, the amount of ^{51}Cr in the faeces collected during the first four days did not differ from the value for their controls (Table 3) Those animals given $^{51}CrCl_3$ three days after the end of irradiation with a dose of 1 000 rad had a higher faecal excretion of ^{51}Cr ($p < 0\ 001$) than that in the control group (Table 3) Such an increase also occurred in the groups examined 6, 10, 14, 28 and 75 days after

Table 6

Body weight (g) of ten mice before and at varying intervals after abdominal irradiation with 1 000 rad

Mouse No	Body weight (g)						
	Before irradiation	Days after irradiation					
		0	3	6	10	14	28
1	28 5	28 0	27 5	27 0	28 0	28 5	29 0
2	28 0	27 0	27 0	27 0	26 5	26 5	28 0
3	27 0	26 5	26 5	26 5	26.5	27 0	27 0
4	27 5	29 0	29 0	27 5	27 0	27 0	26 5
5	29 5	30 0	30 0	30 0	32 0	32 0	35 0
6	30 0	30 0	29 5	29 5	31 0	31 0	31 0
7	28 0	28 0	28 0	29 0	31 0	31 0	31 0
8	28 0	28 0	28 0	27 5	28 0	29 0	30 0
9	27 0	26 0	26 5	27 0	28 0	29 0	30 5
10	27 0	26 0	26 0	26 0	27 0	27 0	28 0

Table 7

^{51}Cr in urine collected on first four days after intravenous injection of the isotope given three days after the end of irradiation of the mice with a varying total dose

Dose (rad)	Urine excretion (%)			Significance of difference from 0 rad
	n	$\bar{X}$	SD	
0	22	14 8	5 5	
500	19	14 3	4 9	—
1 000	20	15 5	9 3	—
2 000	19	9 3	3 6	—
3 000	18	9 6	3 7	—

the end of irradiation The degree of significance of the differences varied (Table 3) but no significant differences in faecal excretion of ^{51}Cr existed between the various groups of control animals

Weight of faeces Comparisons were made between the total weights of the faeces collected on four days from controls and from mice irradiated with different total doses as well as on different occasions from mice that had been irradiated with a total dose of 1 000 rad The collection of the faeces for four days after injection with different total doses was started three days after delivery of the last fraction of the dose No significant differences in weight of the faeces were recorded between the control animals and the mice irradiated with different total doses (Table 4)

Those mice investigated for variation in the weight of the faeces with the interval after irradiation with 1 000 rad were examined immediately after the last fraction of the dose and at the beginning of the 3rd, 6th, 10th and 14th days for four-day periods These series of mice were compared with their controls No significant difference in weight of faeces was evident between the controls and the series of mice irradiated with a total dose of 1 000 rad and examined during different periods after the end of irradiation (Table 5)

Blood in faeces Faeces from ten mice irradiated with a total dose of 1 000 rad were examined every day for blood None was evident except on one occasion when the faeces from one animal contained traces of blood on the 23rd day after the end of irradiation

Survival of experimental animals Twenty mice that had received a total dose of 1 000 rad were observed for 80 days after the end of treatment None of the

Table 8

^{51}Cr in urine collected on first four days following intravenous injection of the isotope given at varying intervals after the end of irradiation

Days after irradiation	Urine excretion (%)						Significance of difference
	Irradiated			Unirradiated			
	n	$\bar{X}$	SD	n	$\bar{X}$	SD	
0	18	10 0	3 8	7	18 8	4 7	***
3	20	15 5	9 3	22	14 8	5 5	—
6	21	16 2	11 7	9	18 9	4 9	—
10	21	18 9	7 8	10	16 2	5 7	—
14	20	18 8	3 9	10	18 7	5 8	—
28	19	17 0	3 2	10	18 0	3 2	—
75	10	19 7	4 0				

animals died within this period Eighteen animals irradiated with a total dose of 3 000 rad died within 7 to 12 days of the end of the treatment

Change in weight after irradiation Ten mice irradiated with a total dose of 1 000 rad were observed for weight loss after irradiation The animals were weighed twice a week for 28 days Nine animals increased in weight and one decreased Six days after the end of irradiation the animals weighed less than they did before irradiation On the 14th and 28th day, however, their weight was significantly higher than before irradiation ($0\ 05 > p > 0\ 01$) Their weight 10, 14 and 28 days after irradiation was also significantly higher than immediately after irradiation ($0\ 05 > p > 0\ 01$) for all of the above mentioned differences (Table 6)

Table 9

^{51}Cr in faeces collected from the second to the fourth day following intravenous injection of the isotope given three days after the end of irradiation of the mice with a varying total dose

Dose (rad	Faecal excretion (%)			Significance of difference from 0 rad
	n	$\bar{X}$	SD	
0	22	2 2	0 8	
500	19	3 2	1 0	—
1 000	20	3 9	1 2	***
2 000	19	4 2	1 3	***
3 000	18	5 0	2 3	***

Table 7

^{51}Cr in urine collected on first four days after intravenous injection of the isotope given three days after the end of irradiation of the mice with a varying total dose

Dose (rad)	Urine excretion (%)			Significance of difference from 0 rad
	n	$\bar{x}$	SD	
0	22	14 8	5 5	
500	19	14 3	4 9	–
1 000	20	15 5	9 3	–
2 000	19	9 3	3 6	–
3 000	18	9 6	3 7	–

the end of irradiation The degree of significance of the differences varied (Table 3) but no significant differences in faecal excretion of ^{51}Cr existed between the various groups of control animals

Weight of faeces Comparisons were made between the total weights of the faeces collected on four days from controls and from mice irradiated with different total doses as well as on different occasions from mice that had been irradiated with a total dose of 1 000 rad The collection of the faeces for four days after injection with different total doses was started three days after delivery of the last fraction of the dose No significant differences in weight of the faeces were recorded between the control animals and the mice irradiated with different total doses (Table 4)

Those mice investigated for variation in the weight of the faeces with the interval after irradiation with 1 000 rad were examined immediately after the last fraction of the dose and at the beginning of the 3rd, 6th, 10th and 14th days for four-day periods These series of mice were compared with their controls No significant difference in weight of faeces was evident between the controls and the series of mice irradiated with a total dose of 1 000 rad and examined during different periods after the end of irradiation (Table 5)

Blood in faeces Faeces from ten mice irradiated with a total dose of 1 000 rad were examined every day for blood None was evident except on one occasion when the faeces from one animal contained traces of blood on the 23rd day after the end of irradiation

Survival of experimental animals Twenty mice that had received a total dose of 1 000 rad were observed for 80 days after the end of treatment None of the

Table 11

^{51}Cr in urine collected from the second to the fourth day after intravenous injection of the isotope given three days after the end of irradiation of the mice with a varying total dose

Dose (rad)	Urine excretion (%)			Significance of difference from 0 rad
	n	$\bar{x}$	SD	
0	22	5 0	1 8	
500	19	4 5	2 0	—
1 000	20	6 1	3 7	—
2 000	19	2 7	0 9	*
3 000	18	3 3	1 8	—

tion of the group examined 6 and 28 days after the injection (Table 10) Analyses of the loss of ^{51}Cr in the urine during the second to fourth day revealed that only in animals treated with 2 000 rad the excretion of the radioisotope was significantly lower than before irradiation (Table 11) Analysis of loss of ^{51}Cr in the urine, as measured from the second to fourth day after injection of the radioisotope at different intervals from the end of irradiation with 1 000 rad disclosed that the excretion of the isotope on the 10th day was significantly lower in the experimental animals than in their controls ($0 01 > p > 0 001$) On the 14th day after the end of irradiation the urinary excretion of the tracer was significantly lower in the controls than in the experimental animals ($0 05 > p > 0 01$) (Table 12) Of the control groups, the one

Table 12

^{51}Cr in urine collected from the second to the fourth day following intravenous injection of the isotope given at varying intervals after the end of irradiation

Days after irradiation	Urine excretion (%)						Significance of difference
	Irradiated			Unirradiated			
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	
0	18	3 8	1 6	7	2 6	1 0	—
3	20	6 1	3 7	22	5 0	1 8	—
6	21	5 2	2 8	9	4 9	2 0	—
10	21	4 3	1 3	10	6 4	2 3	**
14	20	5 0	1 3	10	3 9	1 0	*
28	19	4 3	1 3	10	4 7	2 2	
75	10	7 1	2 3				

Table 10

^{51}Cr in faeces collected from the second to the fourth day following intravenous injection of the isotope given at varying intervals after the end of irradiation

Days after irradiation	Faecal excretion (%)						Significance of differences
	Irradiated			Unirradiated			
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	
0	18	3 2	1 1	10	2 0	1 0	**
3	20	3 9	1 2	22	2 2	0 8	***
6	21	3 3	1 2	9	2 6	0 7	—
10	21	2 8	0 9	10	2 1	0 8	*
14	20	3 2	0 9	10	2 4	0 5	*
28	19	3 1	1 0	10	2 7	0 6	—
75	10	4 1	1 2	7	2 7	0 4	**

Loss of ^{51}Cr in urine In the control animals and the animals irradiated with up to 1 000 rad and examined from the third day after the end of irradiation about 15 per cent of the radioisotope given was excreted during the first four days After irradiation with 2 000 and 3 000 rad the corresponding figure (9 5 per cent) was lower than that for the controls (Table 7) Animals examined immediately at the end of irradiation with 1 000 rad excreted significantly less of the isotope than the control group (Table 8), no difference was otherwise evident between animals irradiated with 1 000 rad and examined at different intervals after the end of irradiation and their respective control groups The average value for the latter usually exceeded 15 per cent but the variation within the groups was so wide that no significant differences between them existed Neither was there any significant difference between the irradiated groups of animals with the exception of the one examined immediately at the end of irradiation, in that group the urinary excretion of ^{51}Cr was lower than that in the other groups of irradiated animals

Loss of ^{51}Cr with faeces and urine during the first 24 hours The differences in the ^{51}Cr content of the faeces from the irradiated animals and from their controls could perhaps be explained by differences in loss of the tracer with the faeces during the first day after the injection All groups were therefore examined regarding the losses in the faeces and urine from the second to the fourth day after the injection The loss of ^{51}Cr with the faeces was significantly higher for the experimental animals irradiated with 1 000 rad or more than for their controls ($p < 0 001$) (Table 9) The values for these animals were also significantly higher at various intervals after the end of irradiation, with the excep-

faeces completely. It may be therefore that part of the urine activity had been included in the faecal samples, which might explain why the faecal excretion of ^{51}Cr proved to be higher for mice than for human beings. Since this risk of contamination of the faeces with urine might have influenced the results, the excretion of the tracer in the present investigation was investigated after the animals had received different doses. As the excretion of ^{51}Cr with urine did not vary with the size of the dose, the higher faecal activity following larger doses cannot be explained by contamination of the urine. No difference in the urine output of ^{51}Cr between the controls and the experimental animals was recorded at different intervals from the end of irradiation, with the exception in those examined immediately following it. The loss of ^{51}Cr was then significantly lower in the irradiated animals than in their controls. The reduction in urine content of the isotope appears to last only for the first day after irradiation, since no such difference was evident during the second to fourth days.

A point of particular interest is whether the increased excretion of ^{51}Cr may be ascribed to gastrointestinal bleeding. ALLEN et coll. (1960) observed superficial lesions in the wall of the stomach and the mucosa of the colon in primates after whole body irradiation with a single dose of 1 500 to 7 500 rad. BARANDUM & WITSCHI (1961) examined mice after whole body irradiation with 500 to 1 000 rad in a single dose. Ten days after irradiation they demonstrated blood in the caecal contents in animals that had received a dose of more than 700 rad. NAKAMURA et coll. (1971) likewise detected blood in the faeces in mice that had received whole body irradiation in a single dose of 750 to 900 rad. The present investigation disclosed no blood in the faeces of animals in which only the abdomen had been irradiated with a total dose of 1 000 rad. The risk of bleeding due to irradiation of the haematopoietic tissue is smaller if only the irradiation be confined to the abdomen and this might explain the discrepancy.

If the total volume of the faeces from irradiated animals during the collection period exceeds that of unirradiated animals, the increased excretion of the tracer may be explained. However, no differences in weight of the faeces between the two groups were evident. All the mice, both the experimental animals and their controls, had been fed the same type of food. None of them had had diarrhoea, so that there was probably no difference in the volume of faeces as evidenced by their weight. DALLA PALMA (1963) reported, however, that the weight of the faeces increased in patients towards the end of radiation treatment of the abdomen, a difference probably explained by the radiation induced diarrhoea.

FRANKENDAL & STIGBRAND (1973) stated that within two hours of the intravenous injection of 10 μCi $^{51}CrCl_3$ in mice about 12 per cent of the dose lay in the low molecular weight part of the plasma, twenty four hours later it was 2 per cent. It is possible that chromium, free or bound to low molecular weight

examined immediately after the injection had lower values than the other control groups (Table 12)

Discussion

Gastrointestinal protein loss after irradiation of the abdomen of mice was estimated from the excretion of ^{51}Cr in the faeces after the intravenous injection of $^{51}CrCl_3$ which unites mainly with the plasma transferrin

The investigation indicated that irradiation of the abdomen in mice was followed by an increased loss of ^{51}Cr in the faeces after irradiation with a total dose of 1 000 rad in two equal fractions at a one day interval An increase in the radiation dose did not, however, produce any further increase in the loss of tracer in the faeces In groups of animals irradiated with 1 000 rad in two equal fractions this loss was significantly higher than in the control groups at different time intervals after the end of irradiation The only exception was that of the group of animals examined immediately after the end of irradiation This is in agreement with the findings of VATISTAS & HORNSEY (1966) in mice after whole body irradiation with a single dose of 300 to 1 200 rad and examined with ^{131}I-polyvinylpyrrolidone These authors reported that the increase of the tracer in the faeces was not demonstrable until 3 to 4 days after the end of irradiation

The present mice irradiated with 1 000 rad survived the observation period (80 days) The dose followed by an increased excretion of ^{51}Cr in the faeces may thus be regarded as sublethal The animals irradiated with 1 000 rad lost weight by six days after the end of irradiation but recovered their original weight within the following week This dose thus had only a mild effect on the general condition of the animal The increased excretion of the tracer in the faeces 75 days after the end of irradiation with a sublethal dose suggests that the gastrointestinal changes may be irreversible FRIEDBERG et coll (1961) examined mice after whole body irradiation and observed the daily albumin loss to be larger than in control animals This difference was demonstrable two weeks after the end of irradiation and persisted throughout the observation period of ten weeks

ROOTWELT (1966) injected 10 to 35 μCi $^{51}CrCl_3$ into ten patients with diseases known not to cause gastrointestinal protein loss and noted that the excretion loss of the tracer in the faeces during the first five days averaged 0 83 per cent of the dose The present investigation in unirradiated mice disclosed an average excretion of 4 to 5 per cent of the tracer in the faeces during the first four days after its injection This difference was probably due to a species difference

When collecting the excreta it was not possible to separate the urine from the

faeces completely It may be therefore that part of the urine activity had been included in the faecal samples, which might explain why the faecal excretion of ^{51}Cr proved to be higher for mice than for human beings Since this risk of contamination of the faeces with urine might have influenced the results, the excretion of the tracer in the present investigation was investigated after the animals had received different doses As the excretion of ^{51}Cr with urine did not vary with the size of the dose, the higher faecal activity following larger doses cannot be explained by contamination of the urine No difference in the urine output of ^{51}Cr between the controls and the experimental animals was recorded at different intervals from the end of irradiation, with the exception in those examined immediately following it The loss of ^{51}Cr was then significantly lower in the irradiated animals than in their controls The reduction in urine content of the isotope appears to last only for the first day after irradiation, since no such difference was evident during the second to fourth days

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FRANKENDAL & STIGBRAND (1973) stated that within two hours of the intra venous injection of 10 μCi $^{51}CrCl_3$ in mice about 12 per cent of the dose lay in the low molecular weight part of the plasma, twenty-four hours later it was 2 per cent It is possible that chromium, free or bound to low molecular weight

substances, may leak into the gastrointestinal canal during the first day after the injection. Determinations were therefore made of the faecal activity exclusive of the values on the first day.

The validity of conclusions concerning leakage of the tracer to the faeces after different doses of radiation and at various intervals after the end of irradiation were not influenced if the values on the first day were excluded. The only exception was the animals examined immediately at the end of irradiation in which the faecal loss of the tracer was higher than in the controls. When the values on the first day were included no significant difference was apparent. This may be explained by the assumption that the loss of protein begins at the earliest on the third day after radiation and that the differences between irradiated animals and the controls is masked by values on the first day. The activity of the urine between the second and fourth day of the injection of a varying dose was the same in the irradiated animals as in the controls during the first four days.

The differences in the urinary excretion of ^{51}Cr at different intervals after the end of radiation with 1 000 rad are difficult to explain. Examinations ten days after irradiation indicated that the excretion of the tracer was significantly higher in the unirradiated animals and fourteen days after irradiation it was lower. This may suggest that between the second and fourth days the amount of tracer excreted was too small to permit of a satisfactory determination.

SUMMARY

A tracer $^{51}CrCl_3$ was injected into female mice irradiated to the abdomen. The faeces and urine were collected and the excretion of the tracer measured. Since ^{51}Cr unites mainly with the plasma protein transferrin, this was taken as a measure of its leakage into the intestine. Mice irradiated with 1 000 rad had a significantly increased leakage to the faeces. They were examined with this technique at various intervals from the end of irradiation: three days later the leakage into the intestine was greater than that in the controls. This increase persisted throughout the experimental period (75 days).

ZUSAMMENFASSUNG

Ein Spurenelement $^{51}CrCl_3$ wurde weiblichen Mäusen nach Bestrahlung des Abdomens injiziert. Faeces und Urin wurden gesammelt und die Ausscheidung des Spurenelements gemessen. Da sich ^{51}Cr hauptsächlich mit dem Plasmaprotein Transferrin verbindet, wurde die Ausscheidung als Mass für die Leckage in den Darm gewertet. Die Mäuse, die mit 1 000 rad bestrahlt waren, hatten eine signifikant gesteigerte Leckage in die Faeces. Die Tiere wurden mit dieser Technik zu verschiedenen Zeitpunkten nach der Bestrahlung untersucht: drei Tage nach Bestrahlung war die Leckage in den Darm höher als bei den Kontrollen. Dieser Anstieg bestand während der Untersuchungsperiode (75 Tage).

RÉSUMÉ

Un marqueur radio-actif, $^{51}CrCl_3$ a été injecté à des souris femelles après l'irradiation du ventre. Les fèces et l'urine ont été recueillies et l'excrétion de ce marqueur a été mesurée. Étant donné que le ^{51}Cr se fixe surtout sur la transferrine protéine plasmatique, cette étude a servi à mesurer sa fuite dans l'intestin. La fuite dans les fèces était considérablement augmentée chez les souris irradiées par 1 000 rad. Ces souris ont été examinées par cette technique à différents intervalles après la fin de l'irradiation: trois jours après la fin de l'irradiation la fuite dans l'intestin est plus grande chez ces souris que chez les témoins. Cette augmentation a duré pendant toute la période expérimentale (75 jours).

REFERENCES

ALLEN R. G., BROWN F. A., LOGIE L. C., ROVNER D. R., WILSON JR S. G. and ZELLMER R. W.: Acute effects of gamma radiation in primates. Radiat. Res. 12 (1960), 532.

BARANDUN S. et WITSCHI H. P.: Étude de la pathogénèse de l'hypoprotéinemie chez la souris irradiée. *In*: Plasma proteins and gastrointestinal tract in health and disease, p. 188. Edited by M. Schwartz and P. Vesin. Munksgaard, Copenhagen 1961.

BIRKE G., JACOBSSON F., LILJEDAHL S. O., PLANTIN L. O. and WETTERFORS J.: Catabolism of albumin and gamma globulin after treatment with ionising radiation to the abdomen. Acta radiol. Ther. Phys. Biol. 6 (1967), 113.

BOND V. P.: Effects of radiation on intestinal absorption. Amer. J. clin. Nutr. 12 (1963), 194.

BROMFIELD A. R. and DYKES P. W.: Radiation induced protein leakage into the small intestine. Nature 201 (1964), 633.

CONARD R. A.: Some effects of ionizing radiation on the physiology of the gastrointestinal tract. A review. Radiat. Res. 5 (1956), 167.

DALLA PALMA L., MASI R. e CIONINI L.: Studio sulla permeabilità della parete intestinale al polivinilpirrolidone J[131] in pazienti irradiati sulla regione addomino-pelvica. (In Italian.) Nunt. radiol. 7 (1963), 531.

EBAUGH JR F. G., CLEMENS JR T., RODNAN G. and PETERSON R. E.: Quantitative measure[illegible]

E[illegible]

[illegible] Foundation. Amsterdam 1968.

FRANKENDAL B. and STIGBRAND T.: Behaviour of intravenously injected $^{51}CrCl_3$ in irradiated and nonirradiated mice. Acta radiol. Ther. Phys. Biol. 12 (1973), 327.

FRIEDBERG W., JENKINS V. K. and MUDERRISOĞLU O. V.: Albumin loss and faecal excretion of polyvinylpyrrolidone in X irradiated mice with implanted rat bone marrow. Int. J. radiat. Biol. 4 (1961), 465.

GRAY S. J. and STERLING K.: The tagging of red cells and plasma proteins with radioactive chromium. J. clin. Invest. 29 (1950), 1604.

HORNSEY S. and VATISTAS S.: Some characteristics of the survival curve of crypt cells of the small intestine of the mouse deduced after whole body X irradiation. Brit. J. Radiol. 36 (1963), 795.

[illegible] Gut leakage [illegible] neutrons and [illegible] Edited by M. [illegible]

JÁNOSSY G.: Intes[illegible] in gastrointestinal irradiation syndrome in the rat. Acta med. acad. Sci. Hung. 26 (1969), 13.

KABAL J BALM S J and PARKHURST L J Canine intestinal vasoactivity during the development of the gastrointestinal radiation syndrome Radiat Res 50 (1972) 528

LOBF J und SCHNEIDER H G Untersuchungen zur enteralen Cr^{51}—Albumin Ausscheidung bei Abdominalbestrahlungen Radiobiol Radiother (Berl) 8 (1967) 47

LUSHBAUGH C C Theoretical and practical aspects of models explaining gastro ntestinal death and other lethal radiation syndromes *In* Comparative cellular and species rad o sensitivity, p 288 Edited by V P Bond and T Sugahara Igaku Shoin Ltd Tokyo 1969

NAKAMURA W, KANKURA T and ETO H Occult blood appearance in feces and tissue hemorrhages in mice after whole body X irradiation Radiat Res 48 (1971) 169

QUASTLER H The nature of intestinal radiation death Radiat Res 4 (1956) 303

ROOTWELT K Direct intravenous injection of ^{51}chromic chloride compared with ^{125}I polyvinylpyrrolidone and ^{131}I albumin in the detection of gastrointestinal protein loss Scand J clin Lab Invest 18 (1966) 405

SULLIVAN M F Intestinal vascular permeability changes induces by radiation or nitrogen mustard Amer J Physiol 201 (1961) 951

VATISTAS S and HORNSEY S Radiation induced protein loss into the gastointestinal tract Brit J Radiol 39 (1966) 547

WALDMANN T A, WOCHNER R D and STROBER W The role of the gastrointestinal tract in plasma protein metabolism Amer J Med 46 (1969) 275

WETTERFORS J LILJEDAHL S O PLANTIN L O and BIRKE G The acute radiation syndrome — the importance of the gastro intestinal injury in the catabolism and distribution of serum albumin Acta med scand 177 (1965) 227

WIERNIK G, CREAMER B and SHORTER R G Recovery of the intestinal mucosa in the rat after X irradiation of the exteriorized intestine Radiat Res 27 (1966) 264

WILLIAMS JR R B, TOAL J N WHITE J and CARPENTER H M Effect of total body X radiation from near threshold to tissue lethal doses on small bowel epithelium of the rat J nat Cancer Inst 21 (1958) 17

FROM RADIUMHEMMET, KAROLINSKA SJUKHUSET, 104 01 STOCKHOLM, AND THE CENTRAL MICROBIOLOGIC LABORATORY, STOCKHOLM COUNTY COUNCIL, 101 22 STOCKHOLM, SWEDEN

EFFECT OF RADIATION TREATMENT ON CELL-MEDIATED IMMUNE RESPONSE IN CARCINOMA OF THE BREAST

ULLA GLAS and J WASSERMAN

The management of the primary treatment of carcinoma of the breast still remains controversial Various investigations have been performed with the purpose of assessing the value of postoperative radiation treatment in combination with prior radical or simple mastectomy According to some reports postoperative radiation treatment decreases the local recurrence of the primary neoplasm but at the same time increases the mortality due to distant metastases (BRUCE 1971, DAO & KOVARIC 1962, FISHER et coll 1970, PATERSON & RUSSELL 1959) It has been suggested that this increased mortality may be caused by an unfavourable effect of the radiation treatment on the immunologic defence mechanism of the patient (MEYER 1970) It may, however, also be due to the problems involved in selecting patients Thus it could be that the patients chosen for postoperative treatment had primarily more advanced carcinomas than those subjected to surgery alone

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In the present investigation patients were divided randomwise into three groups and treated as follows preoperative irradiation followed by surgery, surgery followed by postoperative irradiation and surgery alone The effect of the treatment on the cell-mediated immune mechanisms was then investigated The response of peripheral blood lymphocytes to tuberculin and PHA was chosen as a test indicative of the state of cellular immunity The lymphocyte response to the mitogens was evaluated by determining DNA synthesis by the incorporation of ^{14}C-thymidine This investigation was part of an extensive work on the value and immunologic implications of preoperative and postoperative *radiation therapy as an adjuvant to radical mastectomy in the primary* treatment of operable carcinoma of the breast

Material and Methods

The patients all had untreated operable carcinoma of the breast, the limiting age was 70 and the diagnosis was certified by fine needle aspiration biopsy The patients were examined by a surgeon and a radiotherapist to assure operability and then divided randomwise into three groups The treatment was as follows Group A preoperative irradiation and surgery Group B surgery and postoperative irradiation Group C surgery alone

The postoperative irradiation was directed against the axilla, infra- and supraclavicular regions, both retrosternal regions and the chest wall, the preoperative treatment was applied to the same regions and to the breast The preoperative as well as the postoperative irradiation of the chest wall was planned individually The irradiation was performed with ^{60}Co or high energy electrons

The radical mastectomy was basically the same for all patients The breast and the contents of the axilla were removed en bloc together with the pectoral fascia, but sparing both pectoralis muscles Preoperative radiation therapy was followed within 6 weeks of its completion by operation

The patients were tested according to the following schedule Those belonging to group A (preoperative irradiation and surgery) were examined before any treatment (test A_1), 3 weeks after the completion of the preoperative radiation (A_2) and 3 weeks after the radical mastectomy (A_3)

Patients in group B (surgery and postoperative irradiation) were examined before the operation (B_1), 3 weeks following it (B_2) and 3 weeks after the completion of the postoperative radiation therapy (B_3)

Patients in group C (surgery alone) were examined before the operation (C_1), 3 weeks following it (C_2) and 3 months later (C_3) As a rule the interval between the first and third tests was the same for each group of patients

Table 1

Lymphocyte stimulation in presence of PPD-tuberculin Degree of stimulation expressed as lymphocyte stimulation index (LSI) = CPM (counts per minute) with PPD added CPM without PPD

	Mean	LSI	Statistical significance	
Preoperative radiation therapy				
Before any treatment	A_1	37	$A_1 - A_2$	$p < 0.005$
3 weeks after irradiation	A_2	8		
3 weeks after surgery	A_3	7		
Postoperative radiation therapy				
Before any treatment	B_1	32		
3 weeeks after surgery	B_2	32	$B_2 - B_3$	$p < 0.05$
3 weeks after irradiation	B_3	5		

Lymphocyte stimulation test Some 75 to 100 ml of venous blood from the patients was defibrinated by gentle agitation with glass beads The lymphocytes were isolated by the method of COULSON & CHALMERS (1964, 1966) The cells were washed three times in trisbuffered Hanks balanced salt solution, counted in a hemocytometer, and suspended in Eagle's medium supplemented with glutamine, penicillin and streptomycin The medium also contained 15 per cent of heat inactivated AB serum

A volume of 0 5 ml containing 2×10^6 lymphocytes was pipetted into round bottom 15 ml screw cap tissue culture tubes The mitogens were added to the cells in a volume of 0 5 ml Phytohaemagglutinin (PHA-M, Difco) was used at a final concentration of 0 6 mg/ml, the corresponding concentration for PPD tuberculin (RT 22) being 1 μg/ml, tubes without mitogen served as controls The total volume of the incubation mixture was always 1 0 ml and all concentrations and controls were in triplicate The tubes were loosely closed with screw caps and incubated at 37° C in a humidified 5 per cent CO_2 atmosphere After 72 hours 0 4 μCi of ^{14}C-thymidine (specific activity 54 5 mCi/mM) was added to each tube, which, after a further 24 hours was cooled and washed twice in cooled buffer The washed cells were treated with 5 per cent TCA, dissolved in 0 1 N NaOH and treated again with 6 7 per cent TCA After drying the pellets were dissolved in Soluene TM[100] at 56° C Scintillation fluid was added to aliquots of the dissolved sample and the activity was measured in a Packard Scintillation Counter model 3380

The uptake of ^{14}C-thymidine in each sample was expressed in counts per minute (CPM) and the mean values for the triplicate tubes were calculated The results obtained in the experiments with PHA were expressed as percentages

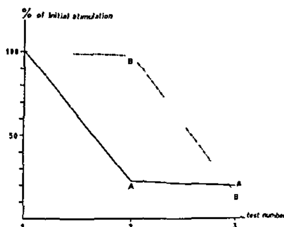


Fig 1 Lymphocyte stimulation in presence of PPD tuberculin Degree of stimulation expressed as lymphocyte stimulation index (I SI) = CPM (counts per minute) with PPD added CPM without PPD All values expressed as percentages of initial values — A—preoperative irradiation B—postoperative irradiation A1 and B1 samples examined before treatment A2 three weeks after preoperative irradiation and A3 three weeks after surgery, B2 three weeks after surgery and B3 three weeks after postoperative irradiation

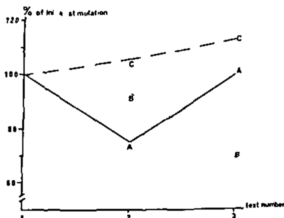


Fig 2 Lymphocyte stimulation in presence of phytohaemagglutinin (PHA) Degree of stimulation expressed as CPM in tubes with lymphocytes in presence of PHA All values as percentages of initial values — A—preoperative irradiation B—postoperative irradiation — — C—surgery alone A1 B1 and C1 samples examined before treatment A2 three weeks after preoperative irradiation and A3 three weeks after surgery B2 three weeks after surgery and B3 three weeks after postoperative irradiation C2 three weeks after surgery and C3 four months after surgery

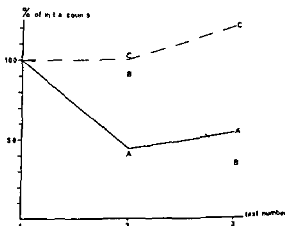


Fig 3 Lymphocyte counts before and after treatment Values expressed as percentages of initial values — A—preoperative irradiation B—postoperative irradiation — — C—surgery alone A1 B1 and C1 samples examined before treatment A2 three weeks after preoperative irradiation and A3 three weeks after surgery B2 three weeks after surgery and B3 three weeks after postoperative irradiation C2 three weeks after surgery and C3 four months after surgery

Table 2

Lymphocyte stimulation in presence of phytohaemagglutinin (PHA). Degree of stimulation expressed as percentage of CPM obtained in the initial tests (A_1, B_1 and C_1)

		Percentage of initial stimulation (mean values)	Statistical significance
Preoperative radiation therapy			
3 weeks after irradiation	A_2	75	$A_2 - A_3$ $p < 0.05$
3 weeks after surgery	A_3	100	
Postoperative radiation therapy			
3 weeks after surgery	B_2	93	
3 weeks after irradiation	B_3	70	$B_2 - B_3$ $p < 0.05$
Surgery only			
3 weeks after surgery	C_2	106	
4 months after surgery	C_3	113	$C_2 - C_3$ Not significant

of the CPM obtained in the initial test for each patient. This was considered to be suitable due to the great variations in PHA response between lymphocytes from different patients. The results obtained in the presence of PPD tuberculin were recorded as the ratio of the CPM with and without antigen. The ratio was termed the 'lymphocyte stimulation index' (LSI). This mode of presentation of the results obtained with PPD was chosen as this mitogen (unlike PHA) is a specific antigen. Consequently the ratio reflects the degree of lymphocyte reactivity in a more meaningful way than by quoting absolute figures. The figures (LSI or percentage of initial CPM) obtained at different times of examination were averaged within each group and subjected to statistical analysis.

Blood for differential counts of white blood cells was obtained in connection with each lymphocyte stimulation test.

Results

Lymphocyte stimulation in the presence of PPD tuberculin. Preoperative as well as postoperative radiation therapy caused a decrease in the reactivity of the peripheral blood lymphocytes, as measured by ^{14}C-thymidine uptake, when PPD tuberculin was used as mitogen. Experiments with lymphocytes from 18 patients belonging to group A and 15 belonging to group B were included in these results.

Table 3

Influence of blood transfusion at the operation on lymphocyte stimulation

	Blood transfusion	No transfusion
PHA M mean CPM	44 276	40 485
PPD mean LSI	9	7

Patients with initial LSI values below 2 were considered as tuberculin negative and of no interest in this context Three tuberculin negative patients, with a mean initial LSI of 1 15, were excluded from group A and 4 patients, with a mean LSI initial value of 1 16, were excluded from group B Table 1 demonstrates the results obtained in the experiments with lymphocytes from the remaining tuberculin positive patients For group A (preoperatively irradiated patients) the degree of stimulation expressed by the lymphocyte stimulation index (LSI) decreased from an initial mean (A_1) of 37 to (A_2) 8 3, three weeks after the completion of the preoperative irradiation This decreased response could still be demonstrated three weeks following the subsequent operation, i e approximately ten weeks after completion of radiation therapy Additionally in group B (postoperative irradiation) the surgical treatment did not have any effect on the reactivity of the lymphocytes The initial mean LSI for group B was (B_1) 32 and 3 weeks after the operation (B_2) was also 32 After the subsequent irradiation the mean stimulation index, however, decreased *to (B_3) 4 9, that is to about the same low level as with the preoperative irradiation* The differences between the mean LSI obtained in experiments performed at times A_1 and A_2 as well as B_2 and B_3 are significant (Table 1) On the other hand the differences between the mean LSI obtained in experiments performed at times A_2 and A_3 or B_1 and B_2 are small and not significant There was no significant difference between the mean initial values of the LSI in both groups (A_1 and B_1)

In Fig 1 the same results are presented with the initial LSI normalized to 100 and all succeeding values expressed as percentages of the initial values The stimulability after irradiation decreased to approximately 20 per cent of the initial values The results obtained in group C were too few to be statistically evaluated

Lymphocyte stimulation in presence of phytohaemagglutinin (PHA) A decrease in the response to stimulation of peripheral blood lymphocytes caused by preoperative and postoperative irradiation could also be demonstrated when phytohaemagglutinin (PHA) was used as mitogen The significance of this

Table 4

Lymphocyte counts before and after treatment

	Mean lymphocyte counts		Statistical significance	
Preoperative radiation therapy				
Before any treatment	A_1	1889	$A_1 - A_2$	$p < 0.005$
3 weeks after irradiation	A_2	833		
3 weeks after surgery	A_3	1009		
Postoperative radiation therapy				
Before any treatment	B_1	1732		
3 weeks after surgery	B_2	1627	$B_2 - B_3$	$p < 0.005$
3 weeks after irradiation	B_3	636		
Surgery only				
Before any treatment	C_1	1686		
3 weeks after surgery	C_2	1702	No significant difference	
4 months after surgery	C_3	1970		

decrease was however not certain (Table 2). The results are summarized in Fig. 2. For group A (21 patients) the degree of stimulation expressed as a percentage of the mean initial CPM decreased 3 weeks after completion of the irradiation from 100 to 75 per cent. Three weeks after the subsequent operation the values became normal. Group B (18 patients) presented no significant decrease of the stimulability after the operation. (The mean value decreased from 100 to 93 per cent.) After the succeeding postoperative irradiation the reactivity of lymphocytes decreased to 70 per cent. There was no significant change in the response to PHA after the operation in lymphocytes of patients belonging to group C (13 patients). The decrease in lymphocyte reactivity after irradiation pre- or postoperatively was significant (Table 2).

During the operation, 7 of the 21 patients included in group A were transfused with whole blood. Lymphocyte reactivity in these patients did not differ significantly from that of other patients in this group, as measured three weeks after the operation (Table 3). Only 3 and 2 patients in groups B and C respectively were transfused.

Lymphocyte counts before and after treatment. Fig. 3 presents the lymphocyte counts before and after the different types of treatment in groups A, B and C. The initial counts are normalized to 100 and the subsequent values expressed as percentages of the initial values for each patient. Preoperative as well as

postoperative irradiation induced lymphopenia On the other hand the operation did not affect the lymphocyte counts The total number of lymphocytes after radiation therapy decreased to 35 to 40 per cent of the initial values, a decrease that is statistically significant (Table 4) The lymphopenia seemed to be of the same order of magnitude and duration as the decreased reactivity to tuberculin

Discussion

The results obtained demonstrated that the responsiveness to tuberculin of lymphocytes from patients with carcinoma of the breast decreased significantly following preoperative as well as postoperative irradiation Neither surgery nor blood transfusion had a similar effect The reactivity to PHA following radiation therapy was also decreased although this phenomenon was less and of shorter duration Experiments performed with sera of irradiated patients and lymphocytes form normal controls revealed that this decreased response to stimulation was due to a changed reactivity of the lymphocyte population and not to some serum factor (BLOMGREN et coll) Thymus derived cells are generally considered to be those which in the first place are stimulated by PHA Measurements of PHA responsiveness have been used as a means to evaluate the potential of cellular immunity in different clinical situations (BROOKS et coll 1972, FISHER et coll 1972, GARRIOCH et coll 1970, HAN & TAKITA 1972, JONES et coll 1971, LEVAN et coll 1969, LEVENTHAL et coll 1969, MCMASTER et coll 1973, SUTHERLAND et coll 1971, WHITTAKER & CLARK 1971) The corresponding stimulation of lymphocytes with specific antigens, e g tuberculin, has been generally regarded as an in vitro reaction specific for cellular immunity to the relevant antigen, indeed it has been reported that lymphocyte stimulation correlates very well with delayed type skin reactions (MILLS 1966, OPPENHEIM et coll 1967) On the other hand evidence has also been presented that lymphocyte stimulation can be related to humoral immunity (BLOCH-SHTACHER et coll 1968) Moreover in case of PPD tuberculin it has been recently revealed that it might act as a B cell mitogen in certain experimental situations (SULTZER & NILSSON 1972) For many reasons, however, it may be assumed that the reactivity to tuberculin presented in the report reflects the cellular immunity to this antigen This does not mean that the cells examined were necessarily those that possessed the effector function On the contrary it is more likely that lymphocytes that react with increased DNA synthesis in the presence of antigen have primarily the recognition function and only after proliferation differentiate into competent effector cells (BLOOM et coll 1972)

Anyway it seems reasonable to assume that the decreased reactivity now reported indicates an impairment of the cellular immune response The simul-

taneous presence of lymphopenia suggests a specific T-cell elimination in agreement with the findings of STJERNSWARD et coll (1972) Another possibility would be a decreased capacity of T-cells to synthetize DNA in the presence of mitogens The present results confirm those reported by MEYER (1970), McCREDIE et coll (1972), and STJERNSWÄRD et coll (1972) that postoperative radiation therapy for mammary carcinoma leads to a decrease in the total number of lymphocytes in the peripheral blood The same has also been observed after radiation therapy to other regions (GOSWITZ et coll 1963) Other authors have also investigated the effect of such therapy on PHA response of lymphocytes from patients with different types of tumours In some of these investigations, as well as in the present report, a decreased response of peripheral blood lymphocytes to PHA has been evident after irradiation (MILLARD 1965, THOMAS et coll 1971) In other experiments, however, no such decrease was present (McCREDIE et coll 1972) The variations in the results may possibly be explained by the differences in the treatment methods as well as in the selection of the patient material The present material in this report was totally unselected, as the patients belonging to different groups were selected randomwise The effects of both the preoperative and postoperative irradiation were examined Finally, the controls were subjects with mammary carcinoma operated upon in exactly the same way as the irradiated lymphocyte donors and not healthy members of the staff or other groups of patients

Another approach to the investigation of the immunologic reactivity of patients with tumours would be to examine the specific immunity to tumour antigens Many such investigations have been performed in vitro with both lymphocyte stimulation and cytotoxicity reactions as assays to evaluate changes in lymphocyte reactivity (HELLSTRÖM et coll 1968, SINKOVICS et coll 1972) The effect of radiation on specific lymphocyte cytotoxicity has also been investigated (O'TOOLE et coll 1972) These authors reported that the specific lymphocyte cytotoxicity in vitro disappeared in the late postirradiation phase of carcinoma of the bladder but reappeared with the recurrence of the malignancy According to these authors the observations suggested that the maintenance of cellular immunity to this neoplasm depends on the presence in the body of critical amounts of tumour material If this applies also to carcinoma of the breast the investigation of specific immunity to tumour cells following irradiation would essentially reflect only the short term success of the treatment On the other hand it would be difficult to draw any conclusion as to the effect of the treatment on cellular immune mechanism in general

In view of the supposed importance of the immunologic surveillance phenomena in cancer patients, the immunologic reactivity might influence the course of disease If so, the information obtained in this and other reports on

the effect of different treatment methods on the immunologic reactivity are certainly valuable It is hoped that this controlled clinical trial in which the groups of patients were selected randomwise, treated by different methods and followed by immunologic tests, will throw more light on this subject

Acknowledgements

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SUMMARY

Patients with carcinoma of the breast were selected randomwise into three groups and treated as follows preoperative irradiation followed by surgery surgery followed by post operative irradiation and surgery alone Peripheral blood lymphocytes from the patients were examined before and after the different types of treatment for their response in vitro to stimulation by PPD tuberculin and phytohaemagglutinin (PHA) Both types of irradiation induced lymphopenia A statistically significant decrease in stimulability by PPD tuberculin and a less evident decrease by PHA were observed after preoperative as well as after postoperative irradiation Surgery did not affect the response of lymphocytes to mitogens or lymphocyte counts The results indicate an impairment of the cellular immune response following irradiation of carcinoma of the breast

ZUSAMMENFASSUNG

Patienten mit einem Karzinom der Brust wurden zufällig in drei Gruppen aufgeteilt und folgendermassen behandelt präoperative Bestrahlung mit nachfolgender Chirurgie Chirurgie mit nachfolgender postoperativer Bestrahlung und Chirurgie alleine Die peripheren Blut lymphozyten dieser Patienten wurden vor und nach den verschiedenen Behandlungsformen hinsichtlich ihrer Antwort in vitro auf Stimulation durch PPD Tuberkulin und Phythaemag glutinin (PHA) untersucht Beide Bestrahlungsformen riefen eine Lymphopenie hervor Ein statistisch signifikanter Abfall der Stimulierbarkeit durch PPD Tuberkulin und ein weniger deutlicher Abfall durch PHA wurden sowohl nach präoperativer als auch nach postopera tiver Bestrahlung gefunden Chirurgische Behandlung beeinflusste die Antwort der Lym phozyten auf die mitogenen Substanzen oder die Lymphozytenzahl nicht Die Ergebnisse deuten auf eine Störung der zellularen Immunantwort nach Bestrahlung eines Karzinoms der Brust hin

RÉSUMÉ

Des malades atteintes de cancer du sein ont été divisées en trois groupes selon qu elles avaient été traitées par les radiations suivies d intervention chirurgicale par intervention chirurgicale suivie de traitement par les radiations ou par intervention chirurgicale seule Les lymphocytes du sang périphérique de ces malades ont été examinés avant et après les différents types de traitement pour apprécier leur susceptibilité in vitro à la stimulation par la tuberculine PPD et par la phytohémagglutinine (PHA) Les deux types d irradiation ont provoqué une lymphopénie Les auteurs ont observé une diminution statistiquement significa tive de la stimulabilité par la tuberculine PPD et une diminution moins évidente par la PHA

aussi bien apres irradiation pre-operatoire qu apres irradiation post-operatoire L intervention chirurgicale n a pas modifié la reponse des lymphocytes aux mitogenes ou a la numération lymphocytaire Les resultats montrent une diminution de la reponse immunitaire cellulaire apres le traitement par les radiations du cancer du sein

REFERENCES

BLOCH SHTACHER N HIRSCHHORN K and UHR J W The response of lymphocytes from non immunized humans to antigen antibody complexes Clin exp Immunol 3 (1968), 889

BLOMGREN H, GLAS U and WASSERMAN J Preliminary unpublished data

BLOOM B R GAFFNEY J and JIMENEZ L Dissociation of MIF production and cell proliferation J Immunol 109 (1972) 1395

BROOKS W H NETSKY M G NORMANSELL D E and HORWITZ D A Depressed cell mediated immunity in patients with primary intracranial tumours J exp Med 136 (1972) 1631

BRUCE J Operable cancer of the breast A controlled clinical trial Cancer 28 (1971), 1443

COULSON A S and CHALMERS G G Separation of viable lymphocytes from human blood Lancet I (1964) 468

— Quantitation of peripheral blood lymphocyte cultures Nature 209 (1966), 378

DAO T L and KOVARIC J Incidence of pulmonary and skin metastases in women with breast cancer who received postoperative irradiation Surgery 52 (1962) 203

FISHER B SAFFER E and FISHER E R Studies concerning the regional lymph node in cancer III Response of regional lymph node cells from breast and colon cancer patients to PHA stimulation Cancer 30 (1972) 1202

— SLACK N H CAVANAUGH P J GARDNER B and RAVDIN R Postoperative radiotherapy in the treatment of breast cancer Results of the NSABP clinical trial Ann Surg 172 1970) 711

GARRIOCH D B GOOD R A and GATTI R A Lymphocyte response to PHA in patients [illegible]

Go[illegible]

HAN T and TAKITA H Immunological impairment in bronchogenic carcinoma A study of lymphocyte response to phytohaemagglutinin Cancer 30 (1972) 616

HELLSTROM I HELLSTROM K E PIERCE G E and YANG J P S Cellular and humoral immunity to different types of human neoplasms Nature 220 (1968), 1352

JONES I H HARDISTY R M WELLS D G and KAY H E M Lymphocyte transformation in patients with acute lymphoblastic leukaemia Brit med J 4 (1971), 329

LEVAN N E KORN C S McNALL E G and PINEDA L Coccidioidomycosis and lymphocyte transformation *In* Proceedings of the third annual leucocyte culture conference Edited by W O Rieke New York 1969

LEVENTHAL B G SMITH C B CARBONE P P and HERSH E M Lymphocyte transformation in response to M pneumonia *In* Proceedings of the third annual leucocyte culture conference Edited by W O Rieke New York 1969

Mc[illegible]

Mc[illegible] 401

MEYER K K Radiation induced lymphocyte immune deficiency A factor in the increased visceral metastases and decreased hormonal responsiveness of breast cancer Arch Surg 101 (1970), 114

MILLARD R E Effect of previous irradiation on the transformation of blood lymphocytes J clin Path 18 (1965) 783

MILLS J A The immunologic significance of antigen induced lymphocyte transformation in vitro J Immunol 97 (1966), 239

OPPENHEIM J J, WOLSTENCROFT R A and GELL P G H Delayed hypersensitivity in the guinea pig to a protein hapten conjugate and its relationship to in vitro transformation of lymph node, spleen thymus and peripheral blood lymphocytes Immunology 12 (1967) 89

O TOOLE C, PERLMAN P, UNSGAARD B, ALMGÅRD L E, JOHANSSON B, MOBERGER G and EDSMYR F Cellular immunity to human urinary bladder carcinoma II Effect of surgery and preoperative irradiation Int J Cancer 10 (1972), 92

PAT

SINI

reactions of patients to cultured cells of breast carcinoma J nat Cancer Inst 48 (1972) 1145

STJERNSWARD J, JONDAL M VANKY F, WIGZELL H and SEALY R Lymphopenia and change in distribution of human B and T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma Lancet II (1972), 1352

SULTZER B M and NILSSON B S PPD tuberculin—a B cell mitogen *In* *Lymphocyte reactivity to PPD tuberculin*, p 65 Edited by B S Nilsson, AB Egnellska Boktryckeriet Stockholm 1972

SUTHERLAND R M, INCH W R and MCCREDIE J A Phytohaemagglutinin (PHA) induced transformation of lymphocytes from patients with cancer Cancer 27 (1971), 574

THOMAS J W LEWIS H S and YUEN A Effect of therapeutic irradiation on lymphocyte transformation in lung cancer Cancer 27 (1971) 1016

WHITTAKER M G and CLARK C G Lymphocyte transformation in patients with breast cancer Brit J Surg 58 (1971) 302

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SPECIAL RADIATION UNITS, SI-UNITS, OR BOTH

KURT LIDÉN

The publication of 'SI units in radiology and radiation measurement' (LIDÉN 1973) was appreciated by the International Commission on Radiation Units and Measurements, ICRU The interest has obviously been awakened by this report, which also appeared in several other journals By October 10, 1973 the ICRU had received about 30 written comments from organizations and individuals Even more comments will no doubt be received in the future

At its meeting in Madrid, 1973, the ICRU devoted much attention to these comments, which displayed a whole spectrum of opinions for and against a conversion to SI units After considerable discussion, the Commission stated in its report to the 13th International Congress of Radiology (Special radiation units, ICRU 1973 a) that for a number of years the ICRU has recommended the use of the International System of Units (SI) although it has continued to recognize and to use the special radiation units—curie, rad, rontgen and rem Recently the International Committee of Weights and Measures (CIPM) has listed the curie, rad and rontgen as temporary units and made no mention of the rem This may denote that CIPM suggests the replacement of special units by those of the International System It should be emphasized that there will be no modification of the current definitions of radiation quantities such as activity, absorbed dose and exposure if this replacement take place

Submitted for publication 16 November 1973

Realizing that such a replacement might result in difficulties as well as advantages the Secretary of the ICRU prepared a document which is published in pertinent journals This document requested opinions and arguments for and against the abandonment of the special radiation units Comments are still being received and must be considered The ICRU will formulate a recommendation on this matter at its July 1974 meeting For the time being, new ICRU Reports will state numerical values of quantities in both SI and special units

In the previous publication (LIDEN 1973) the quantity dose equivalent H and its unit 1 rem were not mentioned This topic has recently been the subject for a special statement from ICRU (ICRU 1973 b), in which it is recognized that dose equivalent H has the same physical dimension as absorbed dose that H can be expressed in $J\ kg^{-1}$, and that it is highly desirable, in matters of radiation safety, that H have its own special unit At present 1 rem is the special unit of dose equivalent 1 rem $= 10^{-2}$ $J\ kg^{-1}$ (exactly) A change to the SI unit 1 $J\ kg^{-1} = 100$ rem would then introduce a unit 100 times greater and would also require consideration of a special name for this new unit

The ICRU would greatly appreciate receiving further comments from members and societies of the radiologic community and health physics profession before its meeting in July, 1974 The comments may be sent to prof Kurt Liden Scientific Secretary of ICRU, Department of Radiation Physics University Hospital, S 221 85 Lund, Sweden

REFERENCES

LIDEN K SI units in radiology and radiation measurement Acta radiol Ther Phys Biol 12 (1973) 81

INTERNATIONAL COMMISSION ON RADIATION UNITS AND MEASUREMENTS (ICRU) (a) Report to the International Executive Committee of the Thirteenth International Congress of Radiology Madrid October 1973

— (b) Dose equivalent Supplement to ICRU Report 19 ICRU Publications Washington D C 1973

Articles published in the series

Acta Radiologica Diagnosis Vol 15 March 1974

ABSTRACTS

RÅDBERG C Brain volume in acromegaly An encephalographic investigation Acta radiol Diagnosis 15 (1974), 113

In 30 acromegalic patients the brain volume was investigated from encephalographic measurements A comparison was made with 34 patients with chromophobe adenoma and a control group of 52 patients With statistical factor analysis it was found that patients with acromegaly had a significantly increased volume of the cerebral parenchyma and in patients with chromophobe adenoma it was somewhat decreased The etiology and implications of these observations are discussed

IKKOS D G, NTALLES K, VELENTZAS CH and KATSICHTIS P Cortical bone mass in acromegaly Acta radiol Diagnosis 15 (1974), 134

Morphometric observations of the left second metacarpal bone of 65 acromegalic patients revealed a statistically significant increase in the external diameter and cortical thickness at the middle of the bone, while the internal diameter was unchanged These findings indicate that the cortical bone mass of the metacarpal—and by implication, the total cortical bone mass—is increased in acromegaly

ROSENGREN K Moya moya vessels Collateral arteries of the basal ganglia Malignant occlusion of the anterior cerebral arteries Acta radiol Diagnosis 15 (1974), 145

Enlarged arteries of the basal ganglia (moya moya arteries) acting as collaterals have usually been regarded as part of a specific Japanese disease Similar cases with arterial occlusion also from other countries have however been described in recent years in the literature A case is presented in which such arterial changes were apparently caused by gradual occlusion of the anterior cerebral arteries by a slowly growing tumour

DUNCAN A M Angiographic abnormalities in combined myositis ossificans and digital ischemia of the hand Report of a case Acta radiol Diagnosis 15 (1974), 152

Two manifestations of occupational trauma in a 36 year old cement finisher are presented These include localized myositis ossificans and posttraumatic digital ischemia Localized hypervascularity and areas of venous obstruction were observed, related to the myositis ossificans as well as to arterial narrowing

LUNDERQUIST A Pharmacoangiography of the left gastric artery in oesophageal varices Acta radiol Diagnosis 15 (1974) 157

Pharmacoangiography of the left gastric artery with bradykinin has been used to demonstrate oesophageal varices The method, which is described and discussed, may further reduce the need for lienoportal phlebography in portal hypertension

Vítek J, Hušák A and Vrubel F Functional isotope phlebography of lower extremities Acta radiol Diagnosis 15 (1974), 161

Functional isotope phlebography is a method of assessing normal or insufficient function of the venous system of the leg The procedure was applied in 30 patients predominantly suffering from varicosities Three types of functional curves have been assessed, these correspond to normal and insufficient function of the venous system as a whole and will reveal occlusion when present

Goransson L R and Jonsson K Scintigraphy of the parasternal lymphatics in the rabbit using technetium 99m sulfide colloid Acta radiol Diagnosis 15 (1974), 169

Parasternal scintigraphy with $^{99}Tc^m$ S colloid has been performed in rabbits The colloid was injected on both sides of the xiphoid process Hyaluronidase dissolved in the colloid was the most favourable method for enhancing the resorption

Dalén N and Lamke B Grading of osteoporosis by skeletal roentgenology and bone scanning Acta radiol Diagnosis 15 (1974), 177

An investigation of randomly selected subjects by skeletal roentgenology and bone scanning revealed that the mineral reduction with age was greater in trabecular than in cortical bone The amount of mineral in the oldest female group was especially low in parts of the skeleton with a high incidence of fractures (distal part of radius, proximal part of humerus and the femoral neck) The mineral content of these sites had the greatest correlation with morphologic signs of osteoporosis

Bjork N, Eliasson S, Falk J-E and Henrikson C O Instrument for isotope determination in vivo of mineral content of the jaw bone Acta radiol Diagnosis 15 (1974), 187

A device for measurement of the bone mineral in the jaws is described The transmission of a narrow beam of radiation from an isotope is measured Windows applied to the mucosa on both sides of the object permit the determination of thickness to be made without disturbance from saliva or mobile tissue Roentgenography of the region under examination is made possible and is a feature of the instrument

Efsen F and Fischerman K Angiography in gastric tumours Acta radiol Diagnosis 15 (1974) 193

Preoperative coeliac and superior mesenteric angiographies were carried out in 15 cases of histologically verified gastric tumours No correspondence was evident between the macroscopic and angiographic sizes of a tumour, neither could the resectability be estimated by angiography No correlation between the angiographic appearance and the histology type of neoplasm was apparent

Nordenstrom B and Kumazaki T Aorta heart and lung vessels in idiopathic pulmonary emphysema related to pulmonary function Acta radiol Diagnosis 15 (1974) 198

The size of the heart and of the aorta has been investigated in 143 patients with idiopathic emphysema The cardio aortic index is described and its value in the estimation of the correlation with the various tests of physiologic function discussed

LINDGREN P, SALTZMAN G-F and ZEUCHNER E Intravenous cholegraphy and peroral contraceptives A preliminary report Acta radiol Diagnosis 15 (1974), 217

A preliminary report is given on the side effects observed at intravenous cholegraphy with iodipamide in women who had been taking oral contraceptives over a long period The age and sex distribution for side effects in a large series of cholegraphies is discussed The circulatory effects on administration of ioglycamide and iodipamide to cats pretreated with progesterone were also investigated

PETTERSSON H Carcinoma of the gallbladder A review of 158 cases Acta radiol Diagnosis 15 (1974), 225

A total of 158 patients with carcinoma of the gallbladder were investigated with various radiologic methods during a 15-year period Almost all relevant positive findings were made in a late phase of the disease when the growth had already spread A high incidence of 'porcelain gallbladder' and carcinoma of the gallbladder was demonstrated, and the presence of a 'porcelain gallbladder' was suggested as indication for cholecystectomy Upper gastrointestinal examination gave most information among the routine methods but angiography of the celiac and superior mesenteric arteries appeared to produce even more reliable information, and should perhaps make a relatively early diagnosis possible

LEVÉN H Arthrography of the knee with a modified technique Acta radiol Diagnosis 15 (1974), 237

A modified technique for arthrography with a positive contrast medium is presented. An immobilizing device in addition to fluoroscopy dodging is used to fix and subject the knee-joint to different constant static forces during the exposure

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BONE MINERAL CONTENT OF THE FEMORAL NECK AFTER IRRADIATION

N DALEN and F EDSMYR

Patients with gynaecologic carcinoma undergoing radiation therapy of the pelvis have been reported to have almost twice the usual incidence of fractures of the femoral neck (ALFFRAM 1964, BICKEL et coll 1961, BONFIGLIO 1953, STEPHENSON & COHEN 1956, TATON & LUKAWSKA 1968) With modern high voltage therapy the bone absorption is considerably lower than was previously the rule, individual dose planning applied in recent years has reduced the risk of excessive irradiation

Radiation therapy has been given to some 600 patients with carcinoma of the bladder between 1957 and 1970 at Radiumhemmet Tumour doses of 6 000 to 7 000 rad have been administered over 7 to 8 weeks with Cobalt-60 units and linear accelerators As no fractures of the femoral neck have been reported, the question arises as to whether the mineral content of the femur is changed by radiation, one that was answered by recording the mineral content in 12 patients with carcinoma of the bladder before, during and after such treatment

Methods and Material The bone mineral content of the femoral neck was determined by roentgen spectrophotometry (GUSTAFSSON et coll 1974) A

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Table

Radiation doses, treatment and follow-up times, sex and age for the 12 patients

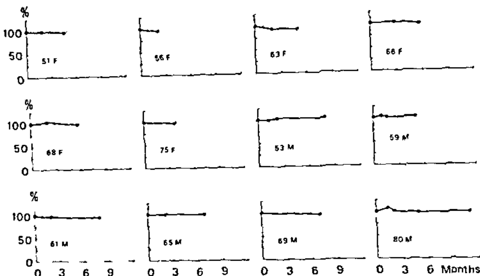
Bladder	Femoral head		Femoral neck	Treatment time, days	Follow-up, months	Sex	Age
Mean tumour dose, rad	Min dose, rad	Max dose, rad	Dose, rad				
4 320	2 800	3 250	< 2 800	42	6	F	51
4 860	1 100	3 200	< 1 100	39	3	F	56
8 400*	1 700	3 100	< 1 700	65	6	F	63
6 480	1 300	2 200	< 1 300	62	6	F	66
4 000	1 600	2 700	< 1 600	29	6	F	69
6 400	1 800	4 300	< 1 800	65	4	F	75
8 400*	1 750	5 000	< 1 750	51	8	M	53
4 000	800	1 900	< 800	28	5	M	59
6 400	2 400	4 500	< 2 400	59	9	M	61
8 100*	3 100	5 500	< 3 100	67	8	M	65
2 800**	2 200	2 200	< 2 200	60	7	M	69
6 480	1 550	4 250	< 1 550	52	12	M	80

* Highly fractionated irradiation: treatment given 3 times daily, 5 days a week, with a rest period of 2 weeks after 4 200 rad.

** Palliative treatment: 2 weeks, with a rest period of 2 months after 1 100 rad.

profile of the mineral content of the part of the skeleton under examination was obtained by scanning with a narrow slit roentgen beam. The absorption by the soft tissues can be compensated for by using two different energies. The site of measurement was the neck of the femur, to which the scanning direction was perpendicular across the narrowest cross-section. The patient lay supine with the legs abducted 35° and the feet inverted 20° to obtain a horizontal position of the neck. The error of the method, as determined by replicate measurements was 1.9 per cent of the mineral content (DALÉN & JACOBSON, in press). The values obtained on each occasion of measurement were expressed as percentages of those recorded before the radiation therapy. The mineral content before treatment for each patient was corrected for age, sex and body size, and the values were compared with the 'normal' values for 170 controls (DALÉN & JACOBSON).

The investigation was performed on 6 women and 6 men ranging in age from 51 to 80 years, mean 64 years. The mineral content was followed for an average of six months, for some patients the period was shorter because of progressive malignant disease (Table).



Variation in the bone mineral content of the femoral neck for each patient with time elapsing after irradiation (The number and the letter, F or M, indicate the age and sex)

An individual dose plan for each patient was drawn up before the radiation therapy was started A Cobalt-60 unit or a 6 MeV linear accelerator was employed The radiation doses to the urinary bladder were varied in accordance with the requirements of clinical experiments that were being conducted with different doses and fractionation schemes The absorbed dose to the femoral head, including the neck, never exceeded 5 500 rad over 67 days, that to the femoral neck was less (Table)

Results

The mean decrease in the bone mineral content for the 12 irradiated patients was only 1 8 per cent, the standard error of the mean was 0 96 The follow-up period after the therapy was 182 days The mean mineral content of the femoral neck for the group before treatment was within the normal range; the mean was only 0 9 per cent less than that for the 170 controls No significant correlation between the decrease in the mineral content and the time elapsing after irradiation Spearman Rank correlation, $p > 0.05$, $r = 0.3$) was evident The change in the mineral content with time for each subject appears in the figure.

Discussion

The mean reduction in the bone mineral content of 1.8 per cent was compared with the normal reduction with age. The regression curves drawn by DALÉN & JACOBSON in a cross-sectional investigation indicated that the corresponding reduction in the mean for the ages and sex distribution now considered here was 0.4 per cent yearly. It is remarkable that the reduction for the patients receiving radiation therapy was not greater in view of their general somatic condition and poor physical state after the treatment. It may be concluded that no essential reduction exists in the bone mineral content for a maximum radiation dose of the order of 2 000 rad.

The high-voltage technique and individual dose planning giving a mean dose of 6 000 rad to the vesical tumour would thus seem to produce no radiation injury to the femoral neck to reduce the mineral content, at least not during the first year after treatment. The clinical course in the 600 patients with carcinoma of the bladder receiving radiation therapy suggest that the femoral neck is immune from injury.

Acknowledgement

This investigation was supported by the Swedish Medical Research Council (Project No. 23X-2580).

SUMMARY

The bone mineral content of the femoral neck has been measured in 12 patients with carcinoma of the bladder before, during and three to twelve months after high-voltage therapy. The mean reduction in the mineral content was only 1.8 per cent, a value that can be ascribed entirely to the normal decrease with age and deficient physical activity following radiation therapy.

ZUSAMMENFASSUNG

Der Knochenmineralgehalt des Femurhalses wurde bei 12 Patienten mit einem Karzinom der Blase vor, während und drei bis zwölf Monate nach einer Hochvoltstherapie bestimmt. Der durchschnittliche Abfall im Mineralgehalt betrug nur 1.8 Prozent, ein Wert, der aus schliesslich auf den normalen Abfall mit dem Alter und auf die fehlende physische Aktivität nach der Strahlentherapie zurückgeführt werden kann.

RÉSUMÉ

La teneur en minéraux du col du fémur a été mesurée chez 12 malades atteints de cancer de la vessie avant, pendant et de trois à douze mois après un traitement par les hautes énergies. La réduction moyenne de la teneur en minéraux a été seulement de 1.8 pour cent, proportion qui peut être attribuée entièrement à la décroissance normale due à l'âge et au défaut d'activité physique consécutif au traitement par les radiations.

REFERENCES

ALFFRAM P A An epidemiologic study of cervical and trochanteric fractures of the femur in an urban population Acta orthop scand (1964) Suppl No 65

BICKEL W H, CHILDS D S and PORRETTA C M Postirradiation fractures of the femoral neck J Amer med Ass 175 (1961), 204

BONFIGLIO M The pathology of fracture of the femoral neck following irradiation Amer J Roentgenol 70 (1953), 449

DALÉN N and JACOBSON B Bone mineral assay Choice of measuring sites Invest Radiol (in press)

GUSTAFSSON L, JACOBSON B and KUSOFFSKY L X ray spectrophotometry for bone mineral determinations Med biol Eng 12(1974), 113

STEPHENSON W H and COHEN B Post irradiation fractures of the neck of the femur J Bone Jt Surg 32 (1956) 830

TATON J and LUKAWSKA K Fractures of the neck of the femur and the pelvis after x ray therapy in cancer of the uterus Polish med J 7 (1968), 965

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RADIATION THERAPY IN THE TREATMENT OF KELOIDS IN EAST AFRICA

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Keloids are particularly common in Africa and because they are often large and invaliding present a serious problem These lesions have hitherto been treated surgically and the frequency of recurrence would seem to have been high (Bourrel et coll 1967) The present investigation was undertaken with the object of examining the results of treatment of keloids in East Africa by surgery or radiation therapy alone, and by surgery followed by irradiation

The series consisted of 142 patients (126 Africans, 11 Asians, 5 Europeans) treated during 1970, 1971 and half of 1972, all with serious symptoms from keloids Seventy-nine of them (73 Africans and 6 Asians) with a total of 138 keloids were controlled for at least 2 months after the completion of treatment by surgery only (12), radiation therapy only (17), excision and postoperative radiation therapy (103) and no treatment (6) The follow-up periods and number of keloids were 2 to 6 months 54 keloids, 6 to 12 months 51 keloids, and over 12 months 33 keloids The irradiation was administered by a roentgen therapy unit (factors 45 and 100 KV, 0 55 and 1 70 mm Al, and 10 and 8 mA) The suture and needle holes were included in the treatment field with a margin of 0 5 cm The total dose varied from 500 to 2 400 R over 1 to 14 days, so that a suitable basis for evaluating it with the results could be obtained For the same reason the time between the operation and the radiation therapy varied from 1 to 22 days

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Table 1

Frequency of recurrence of keloids according to dose in R 1 to 22 days after excision

Dose R days	No of keloids	Recurrences	
		No	Per cent
0	12	12	100
500 – 600/1	6	2	(33)
800 – 1000/1	17	9	53
1200 1	53	6	11
1400 1600 1 – 3	14	0	0
1000 – 1200/7 – 14	4	4	(100)
1600 – 1800 13 – 14	2	0	0
2000 – 2400/2 7	7	0	0

Table 2

Analysis of failures with recurrence after postoperative radiation therapy for keloids

Dose R days	Interval between excision and radiation therapy days	Follow up period months	Comments
500 1	32	13	Low dose long interval
600 1	5	3	Low dose long interval
800 1	21	18	Low dose long interval
800 1	21	18	Low dose long interval
800 1	10	2	Low dose long interval
800 1	10	2	Low dose long interval
800 1	1	6	Low dose
1 000 1	1	12	—
1 000 1	1	12	—
1 000 1	1	3	—
1 000 1	1	3	—
1 200 1	8	9	Unusually large keloids
1 200 1	1	12	Unusually large keloids
1 200 1	5	22	Curved area to be treated (jaw)
1 200 1	1	9	—
1 200 1	1	4	Unusually large keloid (13 cm)
1 200 1	2	2	Unusually large keloids (23 cm × 10 cm)
1 000 8	14	15	Low dose long interval
1 000 8	14	15	Low dose long interval
1 000 8	14	15	Low dose long interval
1 200 14	14	10	Low dose long interval

Table 3

Effect of radiation therapy alone for keloids

Dose, R/days	No of keloids	Follow up period, months	Results
800/1	2	4	Diminished*
800/1	1	3	No effect
1 200/1	3	3	Diminished
1 200/1	4	10	Diminished
2 000/3	1	8	Diminished
2 400/2	2	4	Total regression
2 400/4	3	2	Diminished
2 400/4	1	3	Diminished

* *Objective reduction of keloid and amelioration of symptoms*

Results

All 6 of the keloids in patients not receiving treatment throughout the observation period progressed The patients with the 12 keloids for which the sole treatment consisted in excision had a recurrence usually within 2 months

Eighty-two (80 per cent) of the 103 keloids which received postoperative irradiation did not recur during the follow-up time The frequency of recurrence in relation to the radiation dose appears in Table 1 Since factors other than that of the dose may be of significance for the effect of the postoperative radiation therapy, an analysis of the 21 failures was made (Table 2)

Excision of keloids alone was invariably followed by recurrence (Table 1) Postoperative doses under 1 000 R, and even as low as 500 to 600 R, could prevent recurrences although a considerable number were recorded even when the whole dose was given at a single treatment (Table 1) Further growth after one postoperative treatment with a dose of 1 200 R was fairly uncommon but when it did occur the keloid had usually been large, or an extensive curved area had been irradiated (Tables 1, 2) Doses of over 1 400 R prevented recurrence (Table 1)

Radiation therapy alone to 17 keloids resulted in total regression in 2 patients and a reduction in the keloid with amelioration of the symptoms in 14 patients The treatment was without effect in one patient (Table 3)

Discussion

The fact that excision without radiation therapy was invariably followed by recurrence does not necessarily mean that keloids cannot be cured by surgery

alone It was however, ethically indefensible to withhold radiation for the sole purpose of increasing the number of patients in this admittedly small group These patients had an additional 24 keloids treated by excision and postoperative irradiation and 21 of these disappeared

This form of combined treatment, excision followed by postoperative irradiation has been recommended by a number of authors as suitable management for keloids VAN DEN BRENK & MINTY (1960) stated that between 1 000 and 1 500 R delivered in a single dose is necessary for the suppression of recurrence after excision GREER & VICKERS (1970) reported no recurrence in 25 lesions after 1 500 to 2 000 R given in fractionated doses after excision, among the 31 recurrences 25 lesions had received less than 1 200 R BROWN & BROMBERG (1963) recommended 1 500 R, 18 (60 per cent) out of 30 patients treated had no recurrence CRAIG & PEARSON (1965) stated that 14 out of 16 keloids in 10 patients were cured after a single dose of 800 R following surgery KING & SALMAN (1970) were successful in 74 per cent of their patients with excision followed by electron beam therapy with 1 000 to 3 000 R None of these series consisted of Africans, however, since keloids obviously tend to occur more commonly among Africans than Europeans, and the frequency of recurrence after surgery alone is greater, it is impossible to apply to the former the experience gained from any European series

The present results are strongly in favour of excision followed by postoperative radiation therapy with a single dose of 1 200 R This dose might be increased by 10 to 20 per cent if the keloid is unusually large

VAN DEN BRENK & MINTY recommended a single dose within 48 hours of surgery GREER & VICKERS divided weekly doses over 4 to 5 weeks, the therapy beginning within 24 hours of surgery BROWN & BROMBERG gave treatment over 7 to 10 days starting within 3 weeks of the excision of the keloid and CRAIG & PEARSON a single dose 48 hours postoperatively KING & SALMAN suggested a single dose of electron beam irradiation on the same day as surgery

There would appear to be no reason to fractionate the treatment with the radiation doses that need to be given in the postoperative treatment of keloids The risk of recurrence would seem to exist the longer the interval between the operation and the radiation therapy (Table 2)

Conclusion

Excision alone in the treatment of keloids in Africa produces extremely poor results. Radiation therapy by itself was sometimes effective but by no means satisfactory its main virtue lies in the objective reduction of the keloid and amelioration of symptoms Excision and postoperative radiation therapy seem to

furnish the best results. The preferred dose is 1 200 R, with some increase in unusually large keloids. Fractionation of the dose is unnecessary and it is probably best to irradiate immediately after the operation.

SUMMARY

Seventy-nine patients (73 Africans and 6 Asians) with 138 keloids have been treated and followed for at least two months after excision alone, radiation therapy alone, the two combined or no treatment of the lesion. Excision and immediate postoperative irradiation provided the best results, excision alone gave extremely poor results. Radiation therapy alone led to the objective reduction of the keloid and amelioration of symptoms although the cosmetic result was inferior to that obtained by combined excision and irradiation.

ZUSAMMENFASSUNG

Neunundsiebzig Patienten (73 Afrikaner und 6 Asiaten) mit 138 Keloiden wurden behandelt und wenigstens zwei Monate nach einer Exzision alleine, einer Strahlentheraphie alleine, einer Kombination beider oder ohne Behandlung der Veranderung nachuntersucht. Exzision und unmittelbare postoperative Bestrahlung fuhrte zu den besten Ergebnissen. Exzision alleine fuhrte zu extrem schlechten Ergebnissen. Strahlentherapie alleine fuhrte zu einer objektiven Verminderung des Keloids und zu einer Verbesserung der Symptome, obwohl das kosmetische Ergebnis schlechter als das bei kombinierter Exzision und Bestrahlung war.

RÉSUMÉ

Soixante-dix-neuf malades (73 Africains et 6 Asiatiques) présentant 138 chéloïdes ont été traites et suivis pendant au moins deux mois apres excision seule, apres traitement par les radiations seul, après association des deux, ou sans traitement de la lésion. L'excision suivie immédiatement d'une irradiation post opératoire donne les meilleurs résultats, l'excision seule donne des résultats tres mauvais. Le traitement par les radiations seul amène une réduction objective de la chéloïde et une amélioration des symptomes bien que le résultat esthétique soit inférieur à celui qu'on obtient par l'association d'excision et d'irradiation.

REFERENCES

BOURREL P., RESILLOT A., COLOMAR R. et PIQUARD B. Douze observations de chéloïdes. Méd. trop. 27 (1967), 534.

BROWN J. R. and BROMBERG J. H. Preliminary studies on the effect of time-dose patterns in the treatment of keloids. Radiology 80 (1963), 298.

CRAIG R. D. and PEARSON D. Early post-operative irradiation in the treatment of keloid scars. Brit. J. plast. Surg. 18 (1965), 369.

GREER J. L. and VICKERS B. Combined surgical and X-ray therapy of keloids. J. La med. Soc. 122 (1970), 107.

KING G. D. and SALMAN F. A. Keloid scars. Surg. Clin. N. Amer. 50 (1970), 595.

VAN DEN BRENK H. A. S. and MINTY C. C. J. Radiation in the management of keloids and hypertrophic scars. Brit. J. Surg. 47 (1960), 595.

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ULTRASTRUCTURE OF ^{90}Sr INDUCED OSTEOSARCOMAS AND EARLY PHASES OF THEIR DEVELOPMENT

A NILSSON, P SUNDELIN and INGER SJODÉN

Osteosarcomas induced by ^{90}Sr are of predominantly osteoblastic or fibroblastic types The majority of the former arise along endosteal linings from fusiform cells in the osteogenic connective tissue and the latter from reticular cells of the medullary cavity (NILSSON 1962) The development of both these types is well known and seems to offer good opportunity to investigate ultrastructural phenomena during the early stages of cellular transformation leading to overt osteosarcomas SUNDELIN & NILSSON (1967) have investigated these events by light microscopy and UV-spectrophotometry and to some extent also by electron microscopy Systematic examination of radiation induced cellular changes on the ultramicroscopical level seem to offer information of importance for the understanding of radiation induced oncogenesis The ultrastructural characteristics of ^{90}Sr induced malignancy in osseous and reticular tissues have therefore been investigated

Material and Methods One hundred CBA male mice, 75 days old, were injected intraperitoneally with 0 8 μCi ^{90}Sr/g body weight In order to collect microscopic bone tumours in different stages of development, a total of 50 mice

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were killed in groups of five animals between 200 and 350 days after the injection of ^{90}Sr The mice were killed by cervical dislocation and the time interval between each group was 15 days Both femurs from each mouse were dissected free and were cut longitudinally along the median plane. One part of the femur was then fixed in Stieve's fluid for light microscopy and the other one in glutaraldehyde for electron microscopy After decalcification in 20 % formic acid conventional light microscopic methods were used and the sections were stained according to the van Gieson method By the aid of these sections, material representing different light microscopic stages of the development of predominantly fibroblastic osteosarcomas were selected for the electron microscopic examination The material was classified according to SUNDELIN & NILSSON (1967), by the light microscopic appearance of the reticular cells as follows

Stage 0	Hypoplastic bone marrow with morphologically normal reticular cells (Fig 1 a).
Stage 1	Hypoplastic bone marrow with swelling and increasing number of reticular cells
Stage 2	Hypoplastic bone marrow with proliferation of reticular cells with the histologic appearance of fibrosis without neoplastic character (Fig 1 b)
Stage 3	Solitary or multiple small buds of tightly packed morphologically malignant cells inside an apparently non-neoplastic fibrous tissue (Fig 1 c)
Stages 4, 5	Fibroblastic osteosarcomas situated completely within the bone marrow, the different stages being distinguished by the size, cellularity and pleomorphism of the tumour (Fig 1 d)
Stage 6	Tumours infiltrating and breaking through the compact bone (Fig 1 e)
Stage 7	Overt, paraosteal tumours

After light microscopic examination of the longitudinal half parts of the femurs, the topographical site of microscopic tumours or their early stages was determined From the counter-part fixed in glutaraldehyde (3 % aqueous solution buffered with Na-Cacodylat 0 067 M, pH 7 4) the corresponding region

Fig 1 a) Stage 0 Aplastic fatty marrow with morphologically normal reticular cells Left femur mouse 244 days after injection of 0 8 μCi ^{90}Sr/g body weight Van Gieson × 400
b) Stage II Intramedullary non neoplastic diffuse proliferation of elongated swollen cells Humerus mouse 212 days after injection of 0 8 μCi ^{90}Sr/g body weight Van Gieson × 400
c) Stage III Solitary neoplastic bud inside a non neoplastic fibroblastic tissue diaphysis left femur mouse 270 days after injection of 0 8 μCi ^{90}Sr/g body weight Van Gieson × 100
d) Stage IV Fibroblastic osteosarcoma almost completely occupying the medullary cavity Femur mouse 308 days after injection of 0 8 μCi ^{90}Sr/g body weight Van Gieson × 400
e) Stage VI Intramedullary fibroblastic osteosarcoma breaking through compact bone (right lower corner) Tibia mouse 218 days after injection of 0 8 μCi ^{90}Sr/g body weight Van Gieson × 400

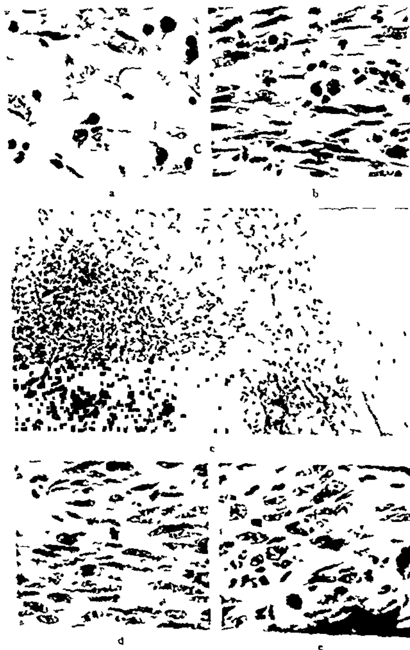


Fig 1 (For legend see opposite page.)

was cut out and postfixed in osmium tetroxide according to MILLONIG (1962) (2 % solution buffered with phosphate buffer, pH 7.3, for one hour at 4° C). The material was dehydrated in ethanol and embedded in Epon (LUFT 1961). One microne sections for survey purposes were stained with 0.2 % toluidin blue (BJÖRKMAN 1962) for light microscopy to detect the relevant piece of tissue. For electron microscopy of this piece sections of about 700 to 900 Å were collected on 200 to 400 mesh copper grids and stained with 6 % uranyl acetate in methanol for 5 minutes (STEMPAK & WARD 1964) and subsequently with 0.2 % lead citrate for one minute (VENABLE 1965). The occurrence of lysosomes was verified with the BARKA & ANDERSSON (1963) method for acid phosphate. The specimens were examined in a Siemen's Elmiscope 1A at 60 kV and at a primary magnification of 1 000 to 20 000. The remaining 50 mice were allowed to survive until macroscopic tumours were detected. Both fibroblastic and osteoblastic osteosarcomas were selected and prepared for light and electron microscopy in the same way as described above.

Results

From the 50 mice killed between 200 and 350 days after ^{90}Sr injection the ultrastructure of 22 femurs with fibroblastic osteosarcomas or typical histologic changes preceding tumour formation were examined. The number of femurs at successive stages were:

Stage	0	1	2	3	4	5	6	7
Number of femurs	3	3	3	2	2	1	3	5

Fibroblastic osteosarcomas

Nuclear structure. The reticular cells of stage 0 had the same appearances as normal reticular cells with elongated nuclei and smooth nuclear membrane without invaginations. The nuclear chromatine had usually a clear light network, which was finely granular and evenly distributed with only slight condensation along the nuclear membrane. Most nuclei contained no perichromatinic granules but in a few cells small clusters of coarse granules were found (Fig. 2).

The nucleoli, one or two per cell nucleus, were small, generally round and granular in type, in some cases with invaginations. No vesicular nucleoli were found.

In cell nuclei from stage I and II—representing a non neoplastic proliferation of reticular cells—the nuclear membrane had a slight tendency to folding and in a few cells also *nuclear budding could occur.* Many cells displayed a marked thickening of the nuclear membrane described as fibrous lamina (FAWCETT 1966) or internal dense lamella (KALIAFAT et coll. 1967) (Fig. 3). The nucleus



F e S age 0 Nu leu w h l el t n wo k of cl oma ne and small clu ers of per h oma ne granu es S el n le sa on f n l a cl roma ne along s n o h nuclear me n rane Sl ghtly d la a ed n ch en p a m e ulu n Gluta ald yde o m um te rox de Epon uranyl a e a e a l ra e 3 000



Fig. 3. Stage II. Fibrous lamination of the nuclear membrane. Left femur, mouse 314 days after injection of [illegible] Sr. Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 32 000



Fig 4 Stage III Electron micrograph from a neoplastic bud of the same type as shown in Fig 1 Femur mouse 317 days after injection of ^{90}Sr The density of the nuclear chromatine varies from cell to cell Strong condensation of chromatine along nuclear membrane and tendency to folding of nuclear membrane Abundant dilated endoplasmic reticulum in some cells Occurrence of fat globules and abundance of lysosome like structures Glutaraldehyde osmium oxide Epon uranyl acetate lead citrate ×4 000

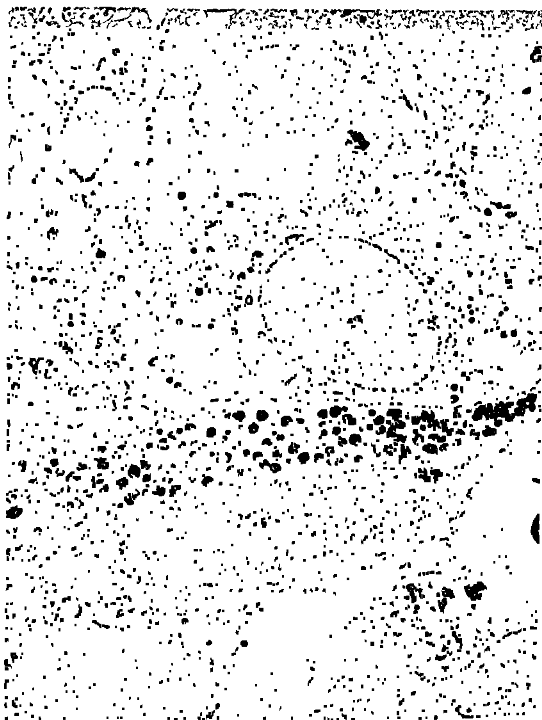


Fig. 5. Stage IV. Chromatine margination less marked but nuclear chromatine more evenly distributed and more finely granular than in earlier stages. In upper part of figure anaplastic cells with scanty cytoplasmic organelles. Abundance of type 4 mitochondria in one cell. Femur, mouse 355 days after injection of ^{90}Sr. Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 4 200.



Fig. 6. Stage 3. Cellular pleomorphism. Numerous cytosomes. Left *femur*, mouse 317 days after injection of ^{90}Sr. Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 4000.



Fig 5 Stage IV Chromatine margination less marked but nuclear chromatine more evenly distributed and more finely granular than in earlier stages In upper part of figure anaplastic cells with scanty cytoplasmic organelles Abundance of type 4 mitochondria in one cell Femur, mouse 355 days after injection of ^{90}Sr Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 4 200



Fig 6 Stage 3 *Cellular pleomorphism* Numerous cytosomes Left femur, mouse 317 days after injection of ^{90}Sr Glutaraldehyde, osmium *tetroxide Epon*, uranyl acetate, lead citrate × 4 000



Fig 7 (For legend see opposite page)

generally appeared slightly more dense than in normal cells and had a coarse network of chromatine which was sometimes clumped into patches Nuclear chromatine also began to condense along the nuclear membrane—in some cases being fairly insignificant, in others, however, evident Accumulations of coarse perichromatine granules were larger and more numerous than had been seen in earlier stages

Cell nuclei from stage III—small circumscript neoplastic condensations of reticular cells displaying polymorphism (Fig 4)—had generally, compared with those from stage I and II, a denser and more finely granular chromatine, although many nuclei also contained greater patches of chromatine material The nuclear infoldings were more numerous and accentuated An evident chromatine margination still persisted in numerous cells

The cell nuclei from stages IV and V—intramedullary tumours—were generally characterized by a strikingly increased cellular pleomorphism and anaplasia (Fig 5) The nuclei were large In many cells the nuclear membrane was more heavily folded than in the preceding stages whereas in others the membrane was very smooth Some of the cells of stage IV had the highest nuclear density seen in the whole material, with an extremely fine granular chromatine The condensation of chromatine along the nuclear membrane had disappeared in many cells but persisted to a varying degree in others (Fig 6) Nucleolar inclusions and invaginations were seen in some of the cells from stage II and III but they were more common in cells belonging to tumours of stage IV and V

The nucleoli from stage VI—penetrating—and VII—overt tumours— were larger than those of earlier stages but their size and shape varied considerably

In these two stages two separate types of cells could be identified (Fig 7) In one of these the cells were anaplastic with large nuclei having smooth margination without folding The nuclear structure was loose with small numbers of granules There was no granular condensation along the nuclear membrane and the pores in the membrane appeared few and narrow The nucleoli of these cells varied considerably in size and shape In the other cell type of stages VI and VII the cells had nucleoli which resembled those of cells from stage V They had dense chromatine, heavily folded nuclear membrane and a varying degree of chromatine condensation (Fig 8)

Cytoplasmic structures The endoplasmic reticulum (ER) was generally of rough type at all stages It was rather scanty in cells of stages 0—II In stage III

Fig 7 Overt fibroblastic osteosarcoma femur mouse 310 days after injection of ^{90}Sr Anaplastic cell in the upper center of the figure with smooth nuclear membrane insignificant chromatine margination and quite loosely arranged chromatine granulation of nucleus poorly developed endoplasmic reticulum hardly detectable Golgi vesicles and few mitochondria but quite abundant polyribosomes (inserted) Glutaraldehyde osmium tetroxide Epon uranyl acetate lead citrate × 5 600 Inserted figure × 16 800



Fig 7 (For legend see opposite page)

the cells appeared with intensely basophilic cytoplasm in the light microscope and electron microscopy revealed abundance of ER in the cytoplasm of nearly all cells from this stage (Fig 4) Cells from stage IV and later disclosed increasing intercellular variation with regard to this parameter Some of the cells from stage VI had more rough ER than any other cells in the whole material These were of the type described above which had dense nuclei with heavily folded nuclear membrane The anaplastic cell type from stage VI and VII tumours had scanty ER which was often degranulated (Fig 7)

Occasionally dilated ER could be found in cells from all stages In cells from stages III and higher it was, however, a regular finding, and cisterns formed by dilated ER often contained dense material (Fig 9)

The mean number of bound ribosomes per cell as estimated in arbitrary units increased gradually from stage 0 to IV and the number of free ribosomes increased from 0 to V In overt tumours the number decreased, but there was an increasing intercellular variation The majority of free ribosomes were polysomes at all stages, but single ribosomes were also found in most cells In anaplastic cells of stages VI and VII, however, the dominance of polysomes was greatest (Fig 7)

The number of mitochondria was counted in 22 to 61 cells at each stage The numbers per cell varied within each stage group but there was a tendency towards increasing numbers from stage 0 to IV and a gradual slight decline in later stages Anaplastic cells of stage VI and VII had few large mitochondria

Lysosomes giving strong reaction for acid phosphate were found in all stages but were especially numerous in stage IV The shape of the Golgi apparatus characterized as mainly tubular, mainly lamellar or mixed and its size was graded from 1 to 3 in each cell There was a great intercellular variation within each stage group and no systematic variation was found when the stage groups were compared In the anaplastic cells of stage VI and VII the Golgi apparatus was usually insignificant In many cells of stage III to VII an abundance of small vesicles in the Golgi zone and its surroundings occurred In the Golgi zone and its immediate surrounding multivesicular bodies (HAGUENAU 1969) were invariably found from stage I to VII They were never numerous and did not evidently predominate a particular stage

Occasionally microtubuli were found in cells from all stages but were most abundant in cells of higher stages

In many cells in the higher intramedullary stages and in overt tumours numerous intracellular fibrils occurred (Fig 10) (ROSS & BENDITT 1965)

Osteoblastic osteosarcomas

The osteoblastic tumours induced by ^{90}Sr originate from the endosteal linings of the bone and they successively fill up the marrow cavity or part of it before

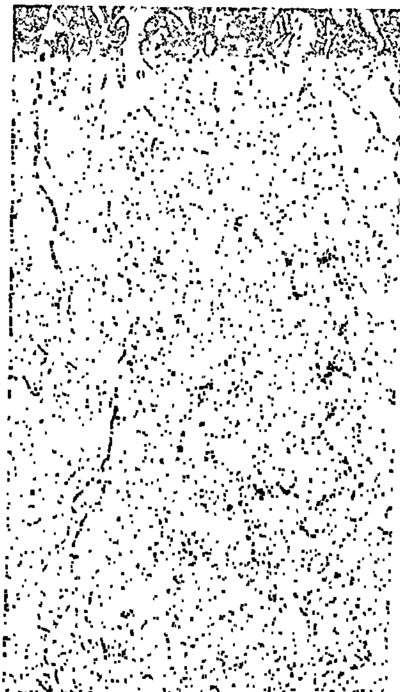


Fig 8 Overt fibroblastic osteosarcoma Heavily folded nuclear membrane (in contrast to Fig 12) and evident chromatine margination Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 16 000



Fig 10. Femur, stage V, mouse 317 days after injection of ^{90}Sr. Numerous intracellular fibrils Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 30 000

Fig 9 Stage IV Femur, mouse 335 days after in[illegible] of ^{90}Sr Abundance of rough endo- [illegible] material Numerous large, elongated [illegible]ct nucleonemata Glutaraldehyde, os-



Fig 9 (For legend see opposite page)

they break through the compact bone They grow slowly and particularly their central parts contain considerable amounts of bone and osteoid tissue Because of their hardness these tumours were difficult to cut in the ultrotome and most material examined originates from peripheral parts of the tumours

Nuclear structure The nuclear membrane was generally heavily folded It had normal thickness and fibrous lamination was never found Nuclear chromatine was finely granular and a great proportion of it was condensed along the nuclear membrane The nucleoli, one or two per cell nucleus, were generally fairly small

Cytoplasmic structures The cytoplasm of osteoblastic osteosarcoma cells contained moderate amounts of ER which was generally of rough type (Fig 11) In many cells dilated ER formed large cisterns, some of which contained electron dense material (Fig 12) The ER was not quite as abundant as in stage V fibroblastic tumours but the cisterns were generally larger in osteoblastic osteosarcomas

Free ribosomes were numerous and a great proportion of them were polysomes

Intracytoplasmic short thread like condensations were found in some cells (Fig 13) Numerous filamentous structures without detectable periodic banding were evident as well as extracytoplasmic collagen fibrils

Regressive changes in these cells were not as marked as in fibroblastic tumours This can be due to their slower growth, their greater vascularization or the fact that the material investigated originates from peripheral parts of the tumours

Discussion

The electron microscopy of the transformation of reticular cells into malignant clones and overt tumours has demonstrated many fine structural changes, some of which, however, seem to be unspecified for the oncogenesis Most of the ultrastructural observations made do not significantly or specifically differ from those observed in tumours induced by other means (see HAGUENAU 1969, BERNHARD 1969)

A clear cellular pleomorphism was identified in stage III and by increasing stage of tumour development it became successively more accentuated During tumour progression the characteristics of the cells differed more and more with respect to every parameter with increasing stage of development In stage VI and VII an increasing population of anaplastic cells were encountered

When examined in the electron microscope single reticular cells of stage 0 did not differ in any specific way from normal reticular cells and no sign of radiation injury could be detected

The earliest ultrastructural changes were found in proliferating cells in stage I in the form of folding of the nuclear membrane In the following stages the

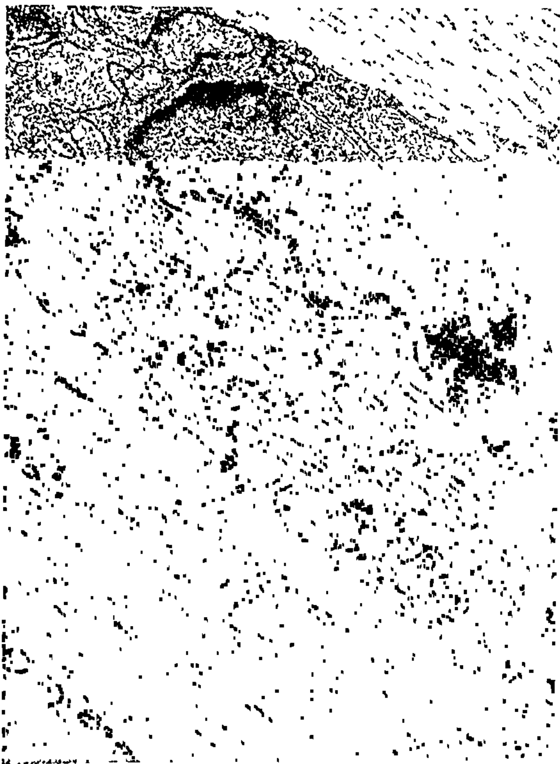


Fig. 11. Overt osteoblastic osteosarcoma 278 days after injection of ^{90}Sr. Well developed dilated rough endoplasmic reticulum. Abundance of ribosomes. Intercellular deposits of collagen. Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 16 000

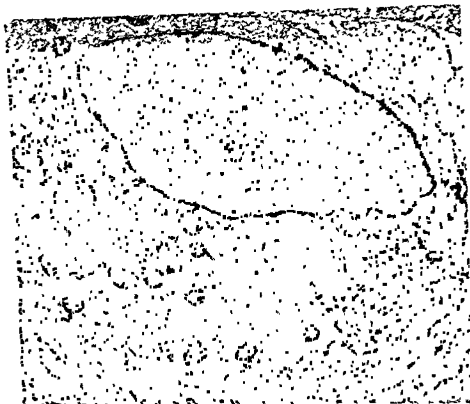


Fig 13 Overt osteoblastic osteosarcoma 237 days *after injection* of ^{90}Sr Great intracellular accumulation of threadlike structures Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 12 000

reticular cells gradually adopted ultrastructural traits which characterize cancer cells hypertrophy of the nucleus and the nucleolus, irregularities in the chromatine distribution, increase in ribosomes—especially polysomes—and increase in number of mitochondria (BERNHARD 1969, HAGUENAU 1969) *etc.*

It is evident that the peak number of mitochondria per cell which is found in stage IV slightly *precedes the* peak number of ribosomes (stage V) and that the peak number of ribosomes *coincides with maximum cytoplasmic ultraviolet*

Fig 12 Overt osteoblastic osteosarcoma 335 days after
Nucleus with deep

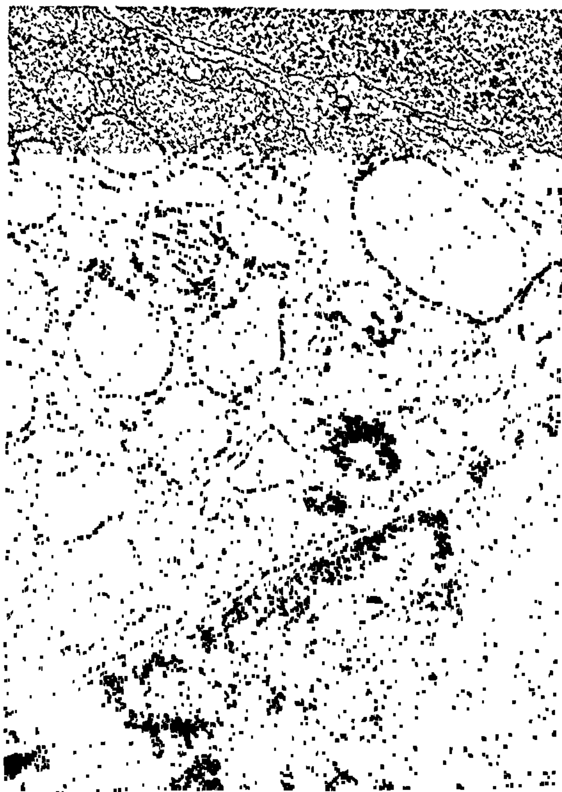


Fig. 12. (For legend see opposite page.)



Fig 13 Overt osteoblastic osteosarcoma 237 days after injection of ^{90}Sr Great intracellular accumulation of threadlike structures Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 12 000

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Fig 12 Overt osteoblastic osteosarcoma 335 days after injection of ^{90}Sr Bottom right Nucleus with deep invagination of nuclear membrane Heavy *chromatine* margination along nuclear membrane *Center left* Strongly dilated rough endoplasmic reticulum Top right Numerous polyribosomes Glutaraldehyde, osmium tetroxide, Epon, uranyl acetate, lead citrate × 32 000

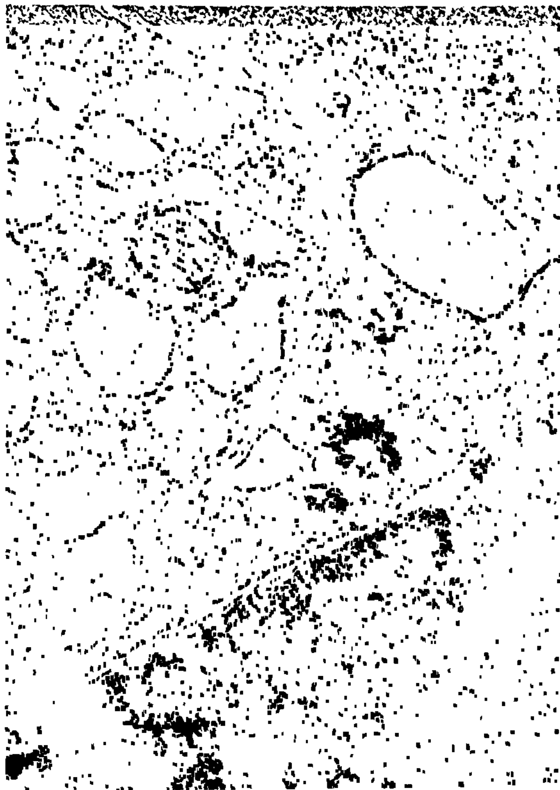


Fig. 12 (For legend see opposite page.)

extinction (SUNDELIN & NILSSON 1967). Also the peak number of lysosome is found in cells around this stage (stage VI). Obviously these changes are reflexions of maximum proliferative activity at stages IV and V. At higher stages the intercellular variation with regard to all these parameters gradually increases.

The nature of the intracytoplasmic fibrillar structures found is not clear but it seems quite plausible to anticipate that these fibrils are identical with those described in fibroblasts by ROSS & BENDITT (1965).

Virus like particles budding from a degranulated part of the endoplasmic reticulum have been found in three separate tumours all of which belonged to stage VII (Figs 14, 15). No virus like particles were found in tumours of earlier stages. As we never have been able to transfer tumours with cell free tumour extract (NILSSON 1962) the possible role of virus in tumourogenesis cannot be stated. The weak transplantation antigenicity which has been found in these tumours (NILSSON & REVESZ 1972) might, however, indicate that the virus is not playing the role as 'driver' but rather as 'passenger', particularly since it also might be anticipated that the antiviral defence inside a tumour is severely impaired thereby creating a 'safe heaven' for many types of virus.

Acknowledgement

This investigation was carried out as part of the programme of the *European Late Effects Project Group* (EULEP).

SUMMARY

The ultrastructure of radiostrontium induced fibroblastic and osteoblastic osteosarcomas and early phases of their development was investigated in CBA mice. The material was classified by light microscopy into eight different stages of development. Fine structural changes were demonstrated such as folding of the nuclear membrane, hypertrophy of nucleus and nucleolus, disturbed chromatine distribution, increase in number of polysomes and mitochondria etc. Virus like particles were found rarely in overt tumours. Their role as driver or 'passenger' is discussed.

ZUSAMMENFASSUNG

Es wurde die Ultrastruktur der durch Radiostrontium induzierten fibrobla[illegible]
osteoblas[illegible]
untersuc[illegible]
lungssta[illegible] veränderungen wie Faltung der Mem-
bran des Nukleus, Hypertrophie [illegible]
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vereinzelt in offenbaren Tumoren [illegible] Passagier' wird
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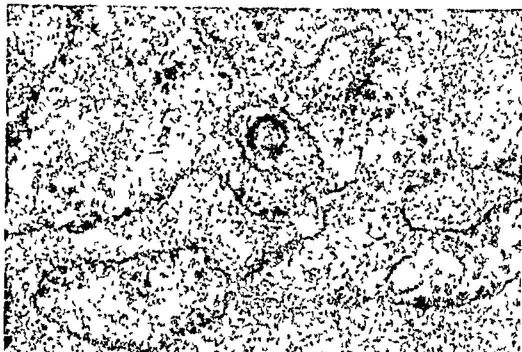


Fig 14 Overt fibroblastic osteosarcoma stage VII from a first generation graft harvested 34 days after transplantation The primary tumour which appeared 311 days after injection of ^{90}Sr was transplanted to new mice subcutaneously Virus like particle inside endoplasmic reticulum Glutaraldehyde osmium tetroxide Epon uranyl acetate lead citrate × 64 000



Fig 15 Same primary tumour as in Fig 13 but harvested 24 days after transplantation Budding of a virus like particle from degranulated part of endoplasmic reticulum Glutaraldehyde osmium tetroxide Epon uranyl acetate lead citrate × 84 000

extinction (SUNDELIN & NILSSON 1967). Also the peak number of lysosome is found in cells around this stage (stage VI). Obviously these changes are reflexions of maximum proliferative activity at stages IV and V. At higher stages the intercellular variation with regard to all these parameters gradually increases.

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ZUSAMMENFASSUNG

Es wurde die Ultrastruktur der durch Radiostrontium induzierten fibroblastischen und osteoblastischen Osteosarkome und die frühen Phasen deren Entwicklung bei CBA Mäusen untersucht. Das Material wurde mittels Lichtmikroskopie in acht verschiedene Entwicklungs[illegible] [illegible]ie Faltung der Membran[illegible] Chromatinverteilung Ans[illegible] [illegible]liche Partikel wurden vere[illegible] Tumoren gefunden. Deren Rolle als Führer oder Passagier wird diskutiert.

RÉSUMÉ

Les auteurs ont étudié l'ultrastructure des ostéosarcomes fibroblastiques et ostéoblastiques induits par le radiostrontium et les premières phases de leur développement sur des souris CBA. Ce matériel a été classé par la microscopie optique en huit stades différents de développement. Les auteurs ont mis en évidence de fines modifications structurales telles que le plissement de la membrane nucléaire, l'hypertrophie du noyau et du nucléole, une perturbation de la distribution de la chromatine, une augmentation du nombre des polysomes et des *mitochondries etc. Ils ont rarement trouvé des particules d'aspect viral dans les tumeurs* évidentes. Ils discutent leur rôle de 'conducteur' ou de 'passager'.

REFERENCES

BARKA, T. and ANDERSSON, P. J.: Histochemistry. Theory, practice and bibliography. Hoeber Medical Division. Harper & Row, New York, Evanston and London 1963.

BERNHARD, W.: Ultra structure of the cancer cell. *In*: Handbook of molecular cytology, p. 688. Edited by A. Lima de Faria. North Holland Publishing Company, Amsterdam-London 1969.

BJORKMAN, N.: Low magnification electron microscopy in histological work. Acta morph. neerl. scand. 4 (1962), 344.

FAWCETT, D. W.: On the occurrence of a fibrous lamina on the inner aspect of the nuclear envelope in certain cells of vertebrates. Amer. J. Anat. 119 (1966), 129.

HAGUENAU, F.: Ultra structure of the cancer cell. *In*: The biological basis of medicine, p. 433. Edited by E. E. Bittar and N. Bittar. Academic Press, London and New York 1969.

LUFT, J. H.: Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9 (1961), 409.

KALIFAT, S. R., BOUTEILLE, M. *et* DELARUE, J.: *Etude ultrastructurale* de la lamelle dense observée au contact de la membrane nucléaire interne. J. Microscopie 6 (1967), 1019.

MILLONIG, G.: Further observations on a phosphate buffer for osmium solutions in fixation. *In*: Proc. 5th Intern. Congr. Electron Microscopy, p. 8. Academic Press, New York 1962.

NILSSON, A.: Histogenesis of ^{90}Sr-induced osteosarcomas. Acta vet. scand. 3 (1962), 185.

— and REVÉSZ, L.: Antigenicity of ^{90}Sr-induced osteosarcomas. Radiat. Res. 52 (*1972*), 395.

O'BRIEN, T. W. and KALF, G. F.: Ribosomes from rat liver mitochondria. II. Partial characterization. J. Biol. Chem. 242 (1967), 2180.

ROSS, R. and BENDITT, E. P.: Wound healing and collagen formation. V. Quantitative electron *microscope radioautographic observations of proline H^3 utilization by* fibroblasts. J. cell Biol. 27 (1965), 83.

STEMPAK, J. G. and WARD, R. T.: An improved staining method for electron microscopy. J. cell Biol. 22 (1964), 697.

SVOBODA, D. J.: Fine structure of hepatomas induced in rats with p-dimethylaminoazobenzene. J. nat. Cancer Inst. 33 (1964), 315.

SUNDELIN, P. and NILSSON, A.: Cytoplasmic ultraviolet extinction of strontium-90 induced fibroblastic osteosarcomas correlated to histologic appearance and ultrastructure. Acta radiol. Ther. Phys. Biol. 7 (1967), 161.

VENABLE, J. H. and COGGESHALL, R. A.: A simplified lead citrate stain for use in electron microscopy. J. cell Biol. 25 (1965), 407.

FROM THE DEPARTMENTS OF GYNECOLOGIC RADIATION THERAPY (DIRECTOR PROF O KJELLGREN) AND DIAGNOSTIC RADIOLOGY (DIRECTOR PROF G-F SALTZMAN), UMEÅ UNIVERSITY, 901 85 UMEÅ, SWEDEN

GASTROINTESTINAL TRANSIT TIME IN OVARIAN CARCINOMA IRRADIATED WITH ^{60}Co

B FRANKENDAL and P JUNGHAGEN

The introduction of high energy radiation has made it possible to deliver larger radiation doses to the abdominal viscera Reaction of the skin previously often limited the dose, while nowadays that of the gastrointestinal tract is one of the main dose limiting factors

Ionizing radiation may impair intestinal peristalsis CONARD (1951) demonstrated disturbances of peristalsis of the small intestine a few minutes after whole body irradiation in the rat He also reported (1956) that the minimum dose necessary to affect peristalsis varied with the species and that animal experiments would therefore not permit any conclusions about the minimum dose producing such alterations in man Only few reports have been published on disturbances of emptying of the stomach and of propulsion in the small intestine following irradiation of the abdomen KORNEEVA (1963) was of the opinion that such treatment accelerated emptying of the stomach WALLACE (1941) investigated the propulsion in the small intestine in 10 patients before and at the end of irradiation of the lower part of the abdomen with 200 or 400 kV for carcinoma of the uterine cervix, in 7 of these the motility was diminished

Submitted for publication 16 March 1973

GUSTERIN et coll (1963) reported intestinal motility to be more or less impaired in most of 74 patients with carcinoma of the uterine cervix treated with intracavitary ⁶⁰Co and external irradiation of the lower part of the abdomen Emptying of the stomach was also temporarily arrested

REEVES et coll (1965) examined 24 patients treated with external irradiation from a cobalt 60 unit to the pelvis and intra uterine radium Examination of the small bowel transit time was carried out on various occasions in the course of the treatment The transit time of the small bowel increased in 5 decreased in 10 and persisted unchanged in the remaining 9 patients

DE MARZI (1965) discussed 68 patients in whom the abdomen and pelvis had been irradiated for various extra intestinal abdominal neoplasms The function of the gastrointestinal tract was examined by photofluorography Irradiation of the pelvis as well as the epigastrium accelerated emptying of the stomach The rate of propulsion of the contents of the small bowel was often increased by the treatment, especially if the latter covered large abdominal fields However their conclusions were not based on statistically significant differences between the rate of propulsion before and during irradiation

Most investigators measured the transit time with barium mixtures LONNERBLAD (1951) stated that the time varied between half an hour and nine hours in 18 to 25 year-old healthy individuals He gave 200 ml barium sulphate mixture, the transit time being calculated from the moment the mixture had been swallowed until it began to appear in the caecum, he also published a survey of the transit times recorded by 20 authors The discrepancies may probably be explained by differences in the examination methods and materials used ERIK (1965) observed that the time calculated from the moment the medium left the stomach till it reached the ileocaecal valve, normally varied between one and four hours PREVÔT (1968) who measured the transit time from the moment the medium passed the duodenojejunal flexure until it reached the ileocaecal valve stated that it varied between two and four hours

The transit time varies with the type of barium meal (MARSHAK & LINDNER 1970) as well as with the volume infused (CALDWELL & FLOCH 1963) LONNERBLAD (1951) stated that emotional factors had no notable effect on the time while BROWN (1959) believed the passage to be accelerated in patients who were nervous at the examination Such diseases as hyperthyroidism Addison's disease and hypoglycaemia accelerated the passage while myxedema hypocalcaemia had a decelerating effect (PREVOT 1968) Diverticulosis and an irritable colon accelerated the passage (MANOUSUS et coll 1967) The time was prolonged in pregnancy (PARRY et coll 1970) Cholecystokinin (JORIFS & MUTT 1969) and metoclopramide (KREEL 1970) proved to stimulate the motility of the small intestine Synstigmine and an ice cold contrast suspension had the same though

less marked, effect as metoclopramide (HOWARTH et coll 1969) Atropine, belladonna alkaloids and morphine prolonged the transit time (CONNELL 1961)

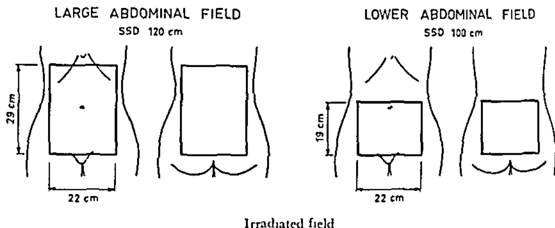
According to LONNERBLAD (1951) and MATTSSON et coll (1960), the rate of emptying of the stomach failed to influence the transit time of the small intestine, TEXTER JR (1968), however, did not agree MATTSSON et coll observed that a mixture of barium sulphate and nutritional substances produced surprisingly uniform rates of gastric emptying and intestinal transit time They mixed two volumes of the barium meal with three volumes of a food component consisting of 60 mg/ml fat, 150 mg/ml carbohydrate and 50 mg/ml protein, the total volume was 300 ml EMBRING & MATTSSON (1966) stated that the barium contrast media commonly used fail to produce any physiologic stimulus of the alimentary canal To obtain more information of the mode of gastric emptying and passage through the small intestine they proposed a standardised test meal, this consisted of 153 g barium sulphate stabilised by trisodium citrate, 12 5 g protein, 15 g fat and 37 5 g carbohydrate, made up to 300 ml by the addition of 200 ml water

The intention of the present investigation was to assess the effect of radiation treatment on the gastrointestinal transit time in patients with ovarian carcinoma The transit time was defined as the interval between the moment the patient had swallowed the contrast medium and the moment it first appeared in the caecum

Material This consisted of 29 patients admitted for ovarian carcinoma All the patients except one had been operated upon for this condition not less than four weeks before the investigation was started All malignant tissue macroscopically visible had been removed at the operation and no mechanical obstruction of the gastrointestinal tract was present The patients had no symptoms or signs of intestinal disorders at the beginning of the examination A further patient aged 49, postmenopausal for somewhat more than a year, had a tumour, almost the size of a hen s egg, close to the left uterine horn Fine needle aspiration biopsy suggested a malignant endometrioid ovarian neoplasm for which the patient received irradiation No symptoms or signs of disturbed peristalsis at the beginning of the irradiation were present, three weeks from the end of treatment she was subjected to bilateral salpingo-oophorectomy Examination of the operation specimen revealed endometriosis, no intra abdominal abnormalities capable of obstructing the gastrointestinal tract had been observed at the operation

The 29 patients aged from 31 to 75 years (median 54 years) were divided into two groups according to the type of medium used, the two groups did not differ significantly from one another in age

Methods The transit time was determined by a standardised roentgen examination The patients had fasted for at least ten hours before the examination,



Irradiated field

which started at 8 a m and at least 16 hours after the last irradiation Sixteen patients were given 200 ml tepid (20° C) barium sulphate suspension (Mixobar, Astra, 0 4 $BaSO_4$ per ml) by mouth, this will be referred to as the conventional contrast medium Thirteen patients received 300 ml tepid medium with nutrients (EMBRING & MATTSSON 1966), this was the physiologic contrast medium

A film of the abdomen was exposed every half hour with the patient prone after the medium had been swallowed until it had begun to pass the ileocaecal valve The patients were allowed between the exposures to move about at will, all these were made in the same laboratory and by the same technician and examined by the same roentgenologist (P. J) The transit time was determined immediately before the beginning of irradiation and repeated when the patients had received a total dose to the abdomen of about 1 000, 2 000, 3 000 and 4 000 rad The treatment was in some cases interrupted for a short period when the patients had received 2 000 rad, the transit time was then again determined immediately before the resumption

The patients were given external beam therapy with a [60]Co kilocurie unit on a standardised schedule Treatment was administered every day, usually on six days a week, the number of irradiations varied between 19 and 29 and the duration of treatment between 25 and 55 days The patients received no drugs known to influence peristalsis during this time although during the first week of the pause in the irradiation series, 4 patients had treatment against diarrhoea

Fourteen patients were given a mid-point dose of about 2 000 rad to the abdomen alternately with large anterior and large posterior fields (Figure) The lower border of the field passed through the symphysis pubis, the upper margin lying at the level of the xiphoid process and the lateral borders at the anterior superior iliac spines, SSD 120 cm The craniocaudal length of the field was about 29 cm The radiation treatment was fractionated, a subcutaneous maximum dose

Table 1

Transit time in minutes with conventional contrast medium

	Case	0 rad	1 000 rad	2 000 rad	2 000 rad after pause	3 000 rad	4 000 rad	Comments
Large + lower abdominal field	1	180	150	120	90	60	60	
	2	120	240	90		90	90	No pause
	3	390	240	240	60	180	210	
	4	90	210	180	120	120	120	
	5	180	90	30	120	240	150	
	6	270	120	150		150	120	No pause
	7	90	90	270	210	120	60	
	8	60	90	120	30	30	30	
Lower abdominal field	9	30	60	30	60	120	30	Clysma before examination at 0 rad
	10	420	180	450		60	60	No pause
	11	210	60	240		120		No pause Treatment ended at 3 000 rad
	12	270	150	90	270	30	90	
	13	150	120	90	90	120		Not examined due to poor general condition
	14	360	360	270	150	210	90	
	15	120	180	90	180	120	60	
	16	240	150	90	120	150	120	

of 200 rad being given in each fraction to produce a mid point dose in the abdomen of about 100 to 150 rad The dose rate in the subcutaneous maximum was about 30 rad/min The treatment was interrupted in 12 patients for about 15 days at a central dose of 2 000 rad and was later on continued against smaller anterior and posterior fields extending from the symphysis pubis to the umbilical planes A SSD of 100 cm and a daily subcutaneous maximum dose of 300 rad gave a central dose to the lower part of the abdomen of about 200 rad a day The dose rate in the subcutaneous maximum was about 45 rad/min An additional mid point dose of 2 000 rad was administered to produce a total central dose to the lower abdomen of about 4 000 rad Eight of these patients were examined with the conventional and 6 with the physiologic contrast medium

The indications for initial irradiation of large abdominal fields in some patients were the opening of the tumour capsule at operation, its penetration by malignant tissue, or microscopic evidence of anaplastic carcinoma A further 15 patients received irradiation confined to the lower contralateral abdominal fields with a total central dose of about 4 000 rad Treatment was temporarily interrupted in 9 of the patients, 8 of these were examined with the conventional and 7 with the physiologic medium Twentyone patients in all had their treatment temporarily interrupted after a dose of 2 000 rad

Statistical method Wilcoxon's t-test was used for comparing the age distribution of patients who received the conventional and physiologic media

The effect of irradiation depends on the size of the dose, the duration of the treatment series and the number of irradiations, wide individual variations forced each patient to serve as her own control The analysis was performed with a parameterfree test although the examination performed immediately after a temporary interruption was not included Five observations per individual were thus the rule, the observation at 0 rad was given the ordinal 1, and the subsequent observations were allotted increasing ordinals The observation at 4 000 rad received the ordinal 5 The values for the transit time were arranged in the same way, the lowest value being given the ordinal 1 and the highest the ordinal 5 The values were indicated in such a way as to produce the smallest possible downward trend when the transit time was the same on different occasions The lowest ordinal subsequently received the lowest value

A null-hypothesis in which it was assumed that the value for the transit time was distributed randomly among the observations was tested against an alternative hypothesis with the transit time tending to vary inversely with the radiation dose The one-tailed alternative hypothesis was based on observations made in animal experiments (FRANKENDAL 1973) and in other clinical investigations (DE MARZI 1965)

The following test was set up

$$t = \sum_{i=1}^{5} i \; t_i$$

t = test variable

i = ordinal

t_i = ordinal for ith observation

For each individual $35 \leqslant t \leqslant 55$

A low t-value for one subject supports the alternative hypothesis

The limit for t in the test was set at 40, which means that the null-hypothesis is confirmed if about 25 per cent of all patients in the material have this or lower

Table 2

Test of hypothesis regarding analysis of changes in transit time when the examination was performed with the conventional medium. Null hypothesis = only random changes of transit time with increasing radiation dose. Alternative hypothesis = trend towards shorter transit time with increasing radiation dose

Dose (rad)	No. of patients		Alternative hypothesis accepted ($p \leq 0.05$)
	t value ≤ 40	Total	
0–4 000	8	13	Yes
0–4 000 (with pause)	6	10	Yes
	t value ≤ 11		
0–2 000	5	15	No
2 000–4 000 (after pause)	5	11	No

values. The table for binominal distribution function will enable the probability (p) afterwards to be calculated for acceptance of the alternative hypothesis if the null hypothesis be true. Since the transit times were arranged as unfavourably as possible for the alternative hypothesis, the true p-value is less than that actually obtained. The alternative hypothesis is accepted for $p \leq 0.05$.

Other limits for t were also used although the conclusions are not dependent on the choice of such a limit. The material was also analysed by the same method but with observations only at 0, 1 000 and 2 000 rad and at 2 000, 3 000 and 4 000 rad. (Only cases in which treatment was temporarily interrupted were included.) The possible ordinals for the observations and transit times were one, two and three, the t value for each individual could be 10 to 14, the limit selected for t being eleven. The effect of the interruption was also analysed with observations immediately before and immediately after the pause, the sign test was used for this analysis (SNEDECOR & COCHRAN 1967).

Results

Conventional contrast medium. The mean transit time for 15 patients before irradiation was 210 minutes, SD 113. A patient who had an enema some hours before the examination was excluded. Fifteen patients irradiated with 4 000 rad were examined, a further patient, taking the same medium, received only 3 000 rad and was also excluded. One of the 15 patients was excluded because she had enema immediately before the first examination and another was not examined at 4 000 rad owing to poor general condition. Of the remaining patients, 3 were examined on five occasions and 10 patients on six occasions depending on

Table 3

Transit time in minutes with physiologic medium

	Case	0 rad	1 000 rad	2 000 rad	2 000 rad after pause	3 000 rad	4 000 rad	Comments
Large+lower abdominal field	1	150	60	60	120	120	30	
	2	90	30	60	60	60	30	
	3	60	60	90	90		60	Examination at 3 000 rad omitted
	4	120	30	30	120	30		Not examined at 4 000 rad due to poor general condition
	5	270	240	180	300	270	240	
	6	60	60	30	90	30	30	
Lower abdominal field	7	90	90	30		60	60	No pause
	8	60	30	60				Refused examination after pause
	9	90	60	30		30	60	No pause
	10	90	60	60		30	60	No pause
	11	60	60	30		30	30	No pause
	12	120	90	30	120	90	60	
	13	180	150	150	240	60	90	

whether treatment had been temporarily interrupted or not. The t-value in 8 of the patients examined was $\leqslant 40$, which weighed against the null-hypothesis and for the alternative hypothesis (Tables 1, 2). This suggests that the transit times decreased significantly in association with irradiation of up to 4 000 rad in the middle of the abdomen.

The findings in the 10 patients irradiated with 4 000 rad with temporary interruption after 2 000 rad were analysed separately. The same conclusion could be drawn, namely that the transit time decreased significantly during irradiation up to a total dose of 4 000 rad.

The changes in transit time during irradiation with 0 to 2 000 rad and with 2 000 to 4 000 rad, respectively, after a pause were analysed. The values obtained failed to confirm any acceleration of the passage (Tables 1, 2). The transit times for examinations immediately before and immediately after the pause were analysed with the sign test; in 6 patients it was shorter after irradiation, in 5 it was longer and in one patient unchanged (Table 1). No significant

Table 4

Test of hypothesis of changes in transit time when the examination was performed with physiologic medium Null hypothesis — only random changes in transit time with increasing radiation dose Alternative hypothesis trend towards shorter transit time with increasing radiation dose

Dose (rad)	No of patients		Alternative hypothesis accepted ($p < 0.05$)
	t value < 40	total	
0 – 4 000	6	10	Yes
0 – 4 000 (with pause)	4	6	No
	t value < 11		
0 – 2 000	11	13	Yes
2 000 – 4 000 (after pause)	6	6	Yes

increase or decrease in transit time after the pause was evident in the present material

Physiologic contrast medium Thirteen patients were examined with the physiologic contrast medium and the mean transit time before irradiation was 111 minutes, SD 60 Three patients were excluded on analysis of the changes in time in association with irradiation with *0 to 4 000 rad* One was in such a poor general condition at 4 000 rad that treatment was discontinued, one refused to participate in the investigation after the pause at 2 000 rad, and one examination at 3 000 rad was omitted The t value was ≤ 40 in 6 of the remaining 10 patients This means *that the transit time became significantly shorter* in association with irradiation to a dose level of 4 000 rad in the abdomen This held true also for irradiation up to 2 000 rad and between 2 000 and 4 000 rad The later analysis included only patients who had been treated with a pause When the analysis was based *only on patients in the group 0 to 4 000* rad who had had a pause in the treatment series no significant shortening of the time could be demonstrated (Tables 3, 4)

The sign test was used for evaluating the transit time before and after the pause In 6 patients it was longer and in 2 patients it was unchanged after the pause, compared with that before This, from a statistical point of view, warrants the conclusion that insertion of a pause prolongs the time

Discussion

LONNERBLAD (1951) reported a mean transit time of 178 minutes in 111 men and women, aged 18 to 25 examined with a 200 ml barium meal KIM (1968) reported on 315 patients examined with barium sulphate He gave no differences

in time with sex or between younger (19 to 39 years) and older (59 to 80 years) patients HOWARTH et coll (1969) examined 50 normals with about 230 ml barium sulphate suspension and found the mean transit time to be 163 minutes CALDWELL & FLOCH (1963) reported a time of 195 minutes in 16 patients examined with eight ounces (236 5 ml) of barium sulphate suspension The mean time in the present material was 210 minutes when the examination was performed with 200 ml barium sulphate suspension No significant difference between the mean times in the present investigation and previous reports was evident

MATTSSON et coll (1960) examined 30 healthy persons with 300 ml physiologic contrast medium The transit time was calculated from the moment the patient had swallowed the medium until it was demonstrable in the caecum, it was 114 minutes This value agrees well with that in the present investigation (111 minutes) with the same volume and with similar, although not identical, physiologic medium Differences between the conventional and physiologic media in transit time may be due to differences in volume (CALDWELL & FLOCH 1963) MATTSSON et coll claimed that since the physiologic contrast medium contains food material it may act as a stimulant to the digestive tract This may also explain the differences in mean transit time

Acceleration of the passage of the physiologic contrast medium was demonstrated at 0 to 2 000, 2 000 to 4 000 and 0 to 4 000 rad Only in the 6 patients in whom the transit time was determined at 0 to 4 000 rad and in whom the treatment series included a pause, was no acceleration evident Since prolongation of the time was demonstrated in this material during the pause, it might explain why no difference was evident for 0 to 4 000 rad with a pause This observation suggests that the radiation induced acceleration disappeared during the pause and returned when the irradiation was resumed With the conventional medium, however, shortening of the time occurred at the dose difference of 0 to 4 000 rad both after continuous treatment and split course treatment On the other hand, no significant decrease in time could be recorded at dose differences of 0 to 2 000 rad and 2 000 to 4 000 rad, neither was any change evident in association with the pause Physiologic contrast medium is considered superior to conventional medium in determining the time (MATTSSON et coll 1960) This might explain the differences between the results obtained with physiologic and conventional contrast media

A parameter-free test was used in the present investigation This was because the material included several variables that could not be properly evaluated The number of irradiations was varied according to the sagittal thickness of the patient The total treatment period was also altered by the number of irradiations and the session free days caused by general holidays and pauses

Differences in the size of the irradiated fields were ignored in the analysis It was assumed in the alternative hypothesis that only quantitative differences in the effect on transit time can be expected between large and lower abdominal fields

Acknowledgement

The authors wish to thank Erik Arvidsson and Torbjorn Linhardt for their help with the statistical analysis

SUMMARY

The gastrointestinal transit time was determined in 29 patients undergoing abdominal irradiation for ovarian carcinoma, 16 patients received 200 ml barium sulphate and 13 patients a 300 ml physiologic contrast meal containing barium sulphate and nutritive substances The transit time was decreased during irradiation and the physiologic meal demonstrated its prolongation during a roughly 15-day pause in the middle of the treatment series

ZUSAMMENFASSUNG

Die gastro intestinale Durchgangszeit wurde bei 29 Patienten, die wegen eines Ovarial karzinoms einer abdominalen Bestrahlung ausgesetzt wurden, untersucht, 16 Patienten erhielten 200 ml Barium Sulphat und 13 Patienten 300 ml einer physiologischen Kontrastmahlzeit bestehend a [illegible] war wahrend der Bestrah [illegible] essen Verlangerung wahren [illegible]

RÉSUMÉ

Le temps de transit gastro intestinal a ete determine chez 29 malades subissant une irradiation abdominale pour cancer de l ovaire, 16 malades ont absorbe 200 ml de sulfate de baryum et 13 malades ont absorbe 300 ml d un repas opaque physiologique contenant du sulfate de baryum et des substances nutritives Le temps de transit diminue pendant l irradiation et le repas physiologique a montre qu'il reste allonge durant environ une interruption de 15 jours au milieu de la serie de traitement

REFERENCES

BROWN F O On routine barium examination of the small bowel Lancet 2 (1959), 530
CALDWELL W L and FLOCH M H Evaluation of the small bowel barium motor meal Radiology 80 (1963), 383
CONARD R A Effect of X irradiation on intestinal motility of the rat Amer J Physiol 165 (1951), 375

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EFFECT OF 200, 650 AND 1 200 R ON THE INTESTINAL DISACCHARASES AND DIPEPTIDASES

A BECCIOLINI, P CARIAGGI, L ARGANINI, P CASTAGNOLI and G DE GIULI

BECCIOLINI & RAVINA (1970) and BECCIOLINI et coll (1972, a, b) have previously followed the activity of certain enzymes of the brush border of the small intestine (JOHNSON 1967, EICHHOLZ 1969) of rats, the abdomen of which had received 500 and 800 R irradiation The enzymes under scrutiny were maltase, invertase, lactase and leucineaminopeptidase (LAP), their activities varying with different time intervals between irradiation and sacrifice To analyze further the dose-effect relationship, the investigation was continued with exposures of 200, 650 and 1 200 R

Material and Methods Ninety female Sprague—Dawley rats, 10 to 12 weeks old and weighing from 150 to 170 g, were employed throughout and were irradiated in groups of four with a Cobalt 60 source as previously described Nine control animals were anaesthetized with sodium pentobarbital (25 mg/kg), and sham irradiated The others, under anaesthesia, were given 200 R (21 rats), 650 R (21 rats) and 1 200 R (39 rats) They were killed in groups of three, at 4, 24, 48 and 72 hours and at 5, 11 and 16 days after irradiation Some animals in the 1 200 R series succumbed to acute intestinal death at 3 to 9 days The

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— Effect of gamma radiation on gastric emptying time in the dog J appl Physiol 9(1956) 234

CONNELL A M The motility of the small intestine Postgrad Med 37 (1961), 703

DE MARZI S Studio radiologico della funzionalità gastro intestinale nei pazienti irradiati sull'addome (In Italian) Radiol med (Torino) 26 (1965) 375

EMBRING G and MATTSSON O An improved physiologic contrast medium for the alimentary tract Acta radiol Diagnosis 4 (1966), 105

FRANKENDAL B Gastric emptying and small intestinal propulsion in mice following irradiation of the abdomen Acta radiol Ther Phys Biol 12 (1973), 529

FRIK W Lehrbuch der Röntgendiagnostik Band V, Abdomen, p 282 Georg Thieme Verlag Stuttgart 1965

GUSTERIN G A, OKULOV N M, STRUTSOVSKAYA S V and BUZINI P A Changes in the gastrointestinal tract during acute radiation sickness in experiments and in patients who received radiation therapy *In* Effects of ionizing radiation on the digestive system p 158 Edited by I F Kurtsin Elsevier publishing company, Amsterdam, London New York 1963

HOWARTH F H, COCKEL R, ROPER B W and HAWKINS C F The effect of Metoclopramide upon gastric motility and its value in barium progress meal Clin Radiol 20 (1969) 294

JORPES J E and MUTT V Clinical aspects of gastrointestinal hormones secretin and cholecystokinin Scand J Gastroent 4 (1969) 49

KIM S K Small intestine transit time in the normal small bowel study Amer J Roentgenol 104 (1968) 522

KORNEEVA V S Changes in gastric secretion and motility following X irradiation of the gastric region *In* Effects of ionizing radiation on the digestive system p 158 Edited by I T Kurtsin Elsevier publishing company, Amsterdam London New York 1963

KREEL L The use of oral Metoclopramide in the barium meal and follow through examination Brit J Radiol 43 (1970) 31

LONNERBLAD L Transit time through the small intestine Acta radiol (1951) Suppl No 88

MANOUSUS O N TRUELOVE S C and LUMSDEN K Transit times of food in patients with diverticulosis or irritable colon syndrom and normal subjects Brit med J 3 (1967) 760

MARSHAK R H and LINDNER A E Radiology of the small intestine p 2 W B Saunders Co Philadelphia London Toronto 1970

MATTSSON O PERMAN G and LAGERLOF H The small intestine transit time with a physiologic contrast medium Acta radiol 54 (1960), 334

PARRY E *SHIELDS R and TURNBULL A C Transit time in the small intestine in pregnancy* J Obstet Gynaec Brit Cwlth 77 (1970) 900

PREVOT R Handbuch der medizinischen Radiologie Vol 11 part 2 p 14 Edited by F Strnad Springer Verlag Berlin 1968

REEVES R J CAVANAUGH P J SHARPE K W THORNE W A WINKLER C and SANDERS A P Fat absorption studies and small bowel X ray studies in patients undergoing ^{60}Co teletherapy and/or radium application Amer J Roentgenol 94 (1965) 848

SNEDECOR G W and COCHRAN W G Statistical methods 6th edition p 120 The Iowa state university press Ames Iowa 1967

TEXTER JR E C Pressure and transit in the small intestine Amer J dig Dis 13 (1968) 443

WALLACE W S The intestine in radiation sickness J Amer med Ass 116 (1941) 583

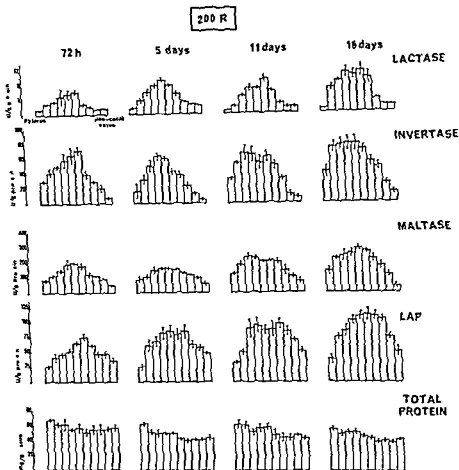


Fig 2 Enzyme activities and total protein content in animals irradiated with 200 R and killed 72 hours and 5 11 and 16 days later

The enzyme activities were expressed in units per *gram of protein* One unit is defined as that which hydrolyses one μ mole of substrate per *minute* at 37° C in the experimental conditions already described The *total* protein content is expressed as mg/g of tissue

The abscissa indicates the ten segments between the *pylorus* and the ileocaecal valve in Figs 1 to 6 The ordinate represents the *mean values* ± standard error (SE) of the enzyme activities and *protein content* for each group of three animals, Fig 7 gives the values for individual animals

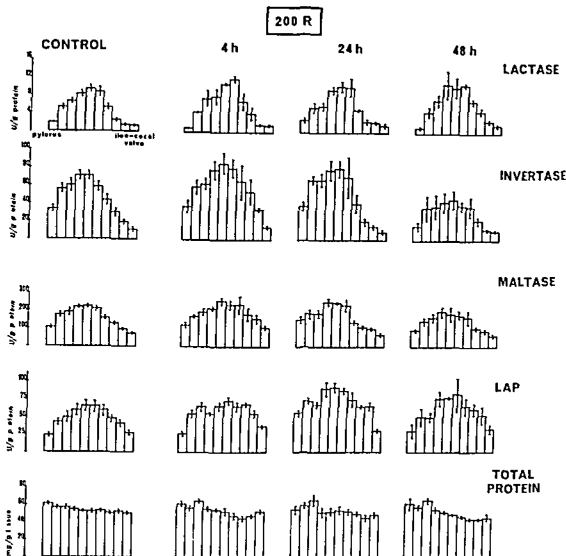


Fig. 1. Enzyme activities and total protein content in control animals and in those irradiated with 200 R and killed 4, 24 and 48 hours later. The ordinate gives the mean values±standard *error (SE) for the ten segments into which the small intestine was divided.*

intestines of these latter rats were examined immediately after death by enzyme activity assays. All the surviving animals were killed later at 48 and 61 days. Three segments, each of one cm, in the proximal and distal parts of the jejunum as well as just above the ileocaecal valve were cut for microscopy. The rest was divided into ten equal segments for determination of enzyme activity. A ten per *cent homogenate (100 mg/ml) of each segment was prepared in cold physiologic* saline (NaCl 0.9 %). The homogenate was centrifuged and the supernatant assayed for enzyme activity (maltase, invertase, lactase and LAP) as previously described. The total protein content was also determined as before.

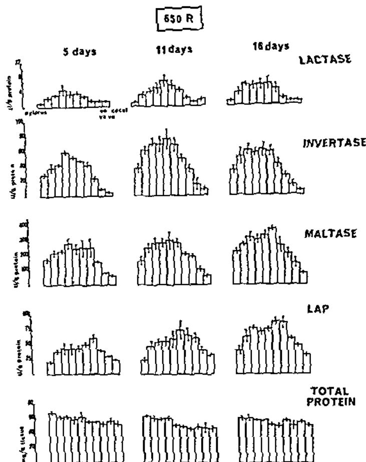


Fig 4 Enzyme activities and total protein content in animals irradiated with 650 R and killed 5 11 and 16 days later

At 5 days (Fig 2) maltase and invertase activities were still reduced but tending towards normal values which they reached after 11 days At 16 days (Fig 2) all enzyme activities, especially LAP, increased

At 4 and 24 hours (Fig 3) after irradiation with 650 R all enzyme activities, especially the maltase and invertase, were increased Lactase was not increased at 4 hours, and LAP presented a much larger increase at 24 hours At 48 hours (Fig 3) all activities, except for the LAP, seemed to be reduced The protein content was slightly reduced At 72 hours (Fig 3) maltase and invertase were the same as at 48 hours Lactase and LAP were reduced while total protein content approached values of non irradiated animals

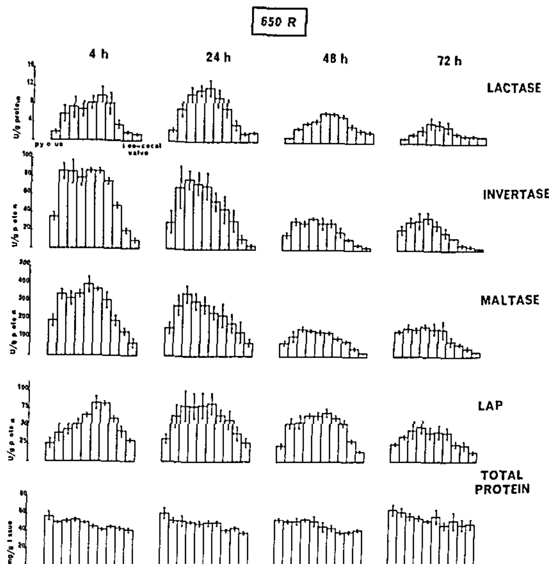


Fig 3 Enzyme activities and total protein content in animals irradiated with 650 R and killed 4 24 48 and 72 hours later

Results

The results obtained with 200 R (Figs 1, 2) together with control values of [illegible] increase in enzyme activities, followed by a reduction phase starting at 48 hours (Fig 1) for maltase and invertase and at 72 hours (Fig 2) for lactase Total protein content at these time intervals slightly reduced

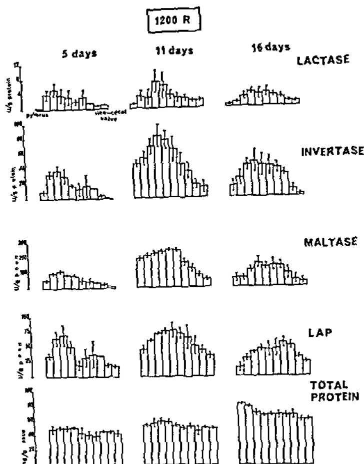


Fig 6 Enzyme activities and total protein content in animals irradiated with 1 200 R and killed 5 11 and 16 days later

the enzyme activities still increased while the total protein content was reduced At 48 hours (Fig 5) marked reduction of all parameters Especially reduced were the maltase, invertase and total protein content, the lactase and LAP were about half the control values At 72 hours (Fig 5) lactase continued to decrease but the invertase, LAP and total protein content had a *tendency* to increase

At 5 days (Fig 6) the enzyme activities although still low, tended to move toward control values, this was particularly so for the upper part of the small intestine In the central zone, where the control values were highest, a reduction of enzyme activities was still apparent At 11 days (Fig 6) an increase in enzyme

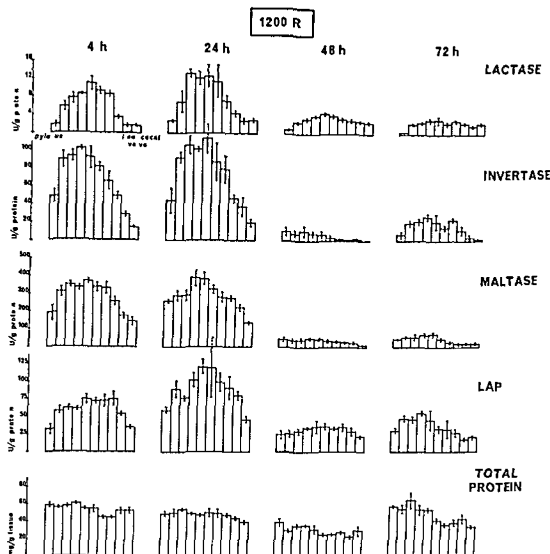


Fig 5 Enzyme activities and total protein content in animals irradiated with 1 200 R and killed 4 24 48 and 72 hours later

At 5 days (Fig 4) lactase and LAP had values much lower than in controls whereas maltase, invertase and protein content levels tended to return to normal At 11 days (Fig 4) considerable increase of enzyme activities over normal values, except for lactase and LAP which were nevertheless higher than at 5 days At 16 days (Fig 4) lactase still reduced whereas maltase and LAP were higher than at 11 days

At 4 hours (Fig 5) with 1 200 R all enzyme activities, especially for maltase and invertase, were higher than those previously reported At 24 hours (Fig 5)

the values for a single rat All values are extremely low and this is particularly evident at 4 days However at 9 days some sectors of the intestine exhibit a certain enzyme activity

The four animals that survived the intestinal reaction and the procedure, were killed later (48 and 61 days after irradiation) They had enzyme profiles varying from normal to grossly reduced activities

Morphologic changes Alterations in morphology were observed with all doses Qualitatively the changes were the same but they varied quantitatively between animals exposed to radiation doses ranging from 200 to 1 200 R

200 R The alterations, although similar to those at higher doses, were small at all time intervals investigated

650 and 1 200 R At 4 hours the alterations occurred in the crypt cells, in some of which lay numerous Feulgen positive fragments which increased in number directly with the dose The mitoses were greatly reduced and became negligible at 1 200 R At 24 hours the alterations in the epithelial cells of the crypts were situated in the proximal parts of the villi, the cells were misaligned, the nuclei being irregular in shape and often enlarged The number of cells was reduced while the mitotic index was greater than at 4 hours At 48 hours the alterations were obvious throughout the epithelium, especially after 1 200 R, the cells themselves were irregular in shape and the villi had lost their individuality, had become flat and consisted of misaligned cuboidal cells The total number of cells and the mitotic index were still lower than in the controls

At 72 hours, after 650 R, signs of recovery were evident in the crypts which however still consisted of misaligned, altered cells After 1 200 R the tendency towards normal morphology was delayed compared to that with 650 R, but the sequence of events was similar, the crypts were first involved and then the villi Normal epithelium was observed at 16 days With smaller doses (200 and 650 R) the epithelium was normal at 5 days after irradiation

The animals that died suddenly following 1 200 R had a loss of epithelium The connective tissue of the lamina propria was haemorrhagic In some rats dying at 9 days after irradiation, focal regeneration of epithelium was noticed The intestinal mucosa appeared normal in the animals killed at 48 and 61 days

All the animals examined had stromal reactions, both of a qualitative and quantitative character, and dependent on the dose The vessels were dilated, with a seepage of blood around them

Discussion

Following some observations on changes after abdominal irradiation (De Gilli et coll 1967, Dalla Palma 1968, Cionini et coll 1971) in absorption of

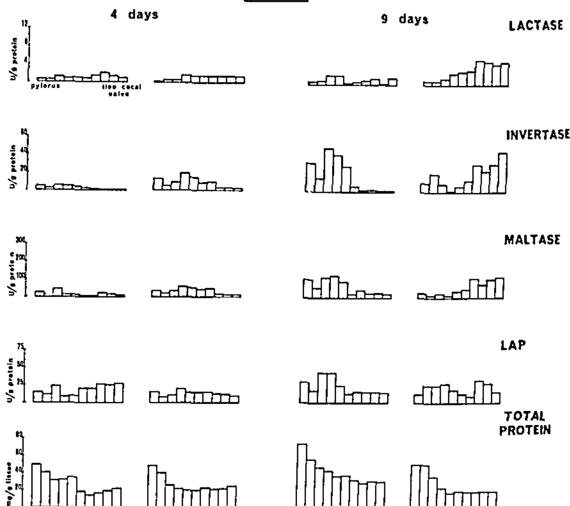


Fig 7 Values for two rats dead at 4 days and for two rats dead at 9 days, the ordinate gives the single value for the ten segments into which the small intestine was divided

activity was evident in all segments of the small intestine The invertase, maltase and LAP were higher than in the controls while the lactase remained reduced At 16 days (Fig 6) all enzyme activities were lower than in the controls and even lower than the values at 11 days It should be noted that for this time interval the total protein content was increased

The 1 200 R irradiation of the abdomen produced such an acute intestinal syndrome that 30 per cent of the animals died between the third and the ninth days following it The enzyme activities were assayed immediately after death to circumvent autolysis phenomena Fig 7 illustrates the values for two rats dead at 4 days and for two rats dead at 9 days Each enzyme activity profile represents

always smaller than for the other enzymes Lactase activity in mammals is high at birth and during lactation but then decreases slowly to reach low values during adult life when its importance is minimal (LITTMAN & HAMMOND 1965)

In conclusion, the present results and those of the previous investigation reveal that in a laboratory animal given a single irradiation to the abdomen (from 200 to 1 200 R), the morphologic alterations in the small intestine already known are associated with changes in enzyme activities, changes that are both time and dose dependent

The initial phase indicates an increase in enzyme activities that is present with all doses and higher with any increase This fact seems to indicate that lesions occur at the level of the enzyme synthesis or at the sites where these enzymes are probably stored in an inactive form, waiting to be transferred in the brush border This could mean the activation of enzymes or the inactivation of inhibitors However, these are still hypotheses since the mechanism of synthesis and transport of these enzymes are unknown

The most relevant results of the present investigation seem to be (1) all exposures even 200 R, produce alterations in enzyme activities of the brush border of a qualitatively similar fashion, (2) these alterations are present throughout the small intestine but their extent is greater in regions where the enzymes are more active in control animals, (3) even at high doses, the surviving animals present more or less a recovery of function This return to normal phase occurs earlier with small doses but is delayed with large doses, (4) the results of microscopy produced insights on the reduction of enzyme activities in the intermediary phase but gave no explanation for the initial and final phases (5) when the various enzymes were compared, their behaviour differed in the timing and extent of changes in activity

Acknowledgements

The authors are grateful to B Jolles for his help with the translation This investigation was supported by the Italian Research Council (C N R.)

SUMMARY

The activity of the brush border enzymes after irradiation with 200 650 and 1 200 R initially increased then decreased and later tended to regain its normal value The three phases were more or less dependent on the magnitude of the dose A correlation between the morphologic and enzymatic data was observed only during the phase of maximum injury

components of diet, post-irradiation changes of disaccharase and dipeptidase activities are described in the present paper

Changes in enzyme activities after 500 and 800 R have previously been reported This investigation has now been extended to single exposures of 200 650 and 1 200 R The 200 R exposure was chosen in an effort to find a threshold dose The 650 R exposure differentiated between the effects already observed with 500 and 800 R Finally the 1 200 R irradiation permitted the examination of the gastrointestinal reaction that was followed in some instances by sudden death

A three-phase sequence was observed for all single doses applied The enzyme activities were higher than in the controls in the initial phase in all animals killed at 4 and 24 hours after irradiation This was followed by an intermediate phase at from 48 to 72 hours in which the enzyme activities as well as the total protein content were reduced A final phase appearing after 5 days was characterized by a return of all parameters to control values

The results described indicate that, even at the 200 R level, changes in enzyme activities are produced although at very much lower levels

The morphologic and enzyme changes after 650 R resemble more the 500 R effects when compared with those caused by 500 and 800 R

The most interesting findings would appear to be those obtained with 1 200 R This amount of irradiation produced an initial phase of greater enzyme increase than that for lower doses In the intermediary phase the nearly complete disappearance of maltase and invertase as previously evident with 800 R was noted, a residual activity of lactase and LAP still persisted Only in the animals killed at 11 days was there a clear increase' phase (even if the activities remained lower than those of the controls) This was with the exception of lactase, which failed to reach control values even after 16 days

Great variability in the animals of each group 48 hours after 1 200 R, due to the severity of the gastrointestinal reaction, occurred Moreover, the return to normal values (11 to 16 days) was not uniform along the whole length of the intestine In some rats examined after 16 days, the digestive function of the epithelium, as determined by the disaccharase and dipeptidase activities, failed to return to normal This reduction took place in some rats even after 48 and 61 days, in spite of a normal mucosa, as judged by its appearances under the microscope

Among other interesting results was the fact that for all doses and at the same time intervals, all enzymes failed to behave quantitatively in the same fashion The reduction in lactase was delayed compared to that in maltase and invertase Furthermore, the lactase was, except for 200 R, still low when the other enzymes had returned to normal levels The reduction in lactase and LAP activities is

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POTENTIAL DOUBLING TIMES OF HUMAN SQUAMOUS CARCINOMAS AS MEASURED BY VINCRISTINE

B J SHEPSTONE, R SEALY, ANITA GREENSTEIN and L F RAPLEY

The group of alkaloids extracted from *Vinca rosea* Linn are at present being extensively investigated on account of their anti tumour and anti mitotic activity Of these alkaloids, vinblastine has received most attention, but more recently vincristine has been shown to possess similar properties

The stathmokinetic effect of vincristine in tissue culture and in vivo has been demonstrated by several authors (CUTTS et coll 1960, CARDINALI et coll 1963, FREI III et coll 1964), and the present report constitutes a further demonstration of this effect in a number of solid human tumours The response of the tumour cells is investigated and an attempt is made to calculate the potential doubling time of the tumour cells

Materials and Methods Twelve patients with advanced squamous cell carcinomas were chosen for the investigation All the tumours were large and painless to biopsy In order to minimize the possible long term risks of a dose of vincristine only patients with short life expectancy were chosen and, wherever possible, they were also elderly

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ZUSAMMENFASSUNG

Es konnte gezeigt werden wie die Aktivität der Enzyme an der Grenze der Cilienbürste nach Bestrahlung mit 200, 650 und 1 200 R anfänglich zunimmt, dann abnimmt und endlich zum Normalwert zurückkehrt Die drei Phasen hängen im allgemeinen von der Dosengrösse ab Ein Zusammenhang zwischen morphologischen Veränderungen und dem Enzymniveau konnte nur während der Periode des Maximalschadens festgestellt werden

RÉSUMÉ

L activité des enzymes des bordures en brosse après irradiation par 200, 650 et 1 200 R a d abord augmenté puis diminué et enfin a tendu à retrouver sa valeur normale Ces trois phases sont plus ou moins dépendantes de l importance de la dose Les auteurs n ont trouvé une corrélation entre les données morphologiques et les données enzymatiques que pendant la phase de perturbation maximale

REFERENCES

BECCIOLINI A and RAVINA A Effect of ionizing radiation on intestinal disaccharidases in rats Brit J Radiol 43 (1970), 150

— — ARGANINI L, CASTAGNOLI P and DE GIULI G (a) Effect of ionizing radiation on the enzymes of the intestinal mucosa of rats at different time intervals after abdominal irradiation Radiat Res 49 (1972), 213

— ARGANINI L and DE GIULI G (b) New aspects of intestinal radiation damage IXth Annual Meeting of the European Society for Radiation Biology, Rome 1972

CIONINI L, BECCIOLINI A, DALLA PALMA L and DE GIULI G Intestinal absorption of radioiodine labelled human serum albumin mono iodotyrosine and di iodotyrosine following abdominal radiation therapy Acta radiol Ther Phys Biol 10 (1971), 341

DALLA PALMA L Intestinal malabsorption in patients undergoing abdominal radiation therapy *In* Gastrointestinal radiation injury, p 261 Edited by M F Sullivan Excerpta Medica Foundation Amsterdam 1968

DE GIULI G, DALLA PALMA L and DE DOMINICIS R The prophylaxis and treatment of intestinal damage due to radiation *In* Proceedings of the XIth International Congress of Radiology p 1609 Excerpta Medica Foundation Amsterdam 1967

EICHHOLZ A Fraction on the brush border Fed Proc 28 (1969) 30

JOHNSON C F Disaccharidase localization in hamster intestine brush border *Science* 155 (1967), 1670

LITTMAN A and HAMMOND J B Diarrhea in adults caused by deficiency in intestinal disaccharidases Gastroenterology 48 (1965) 237

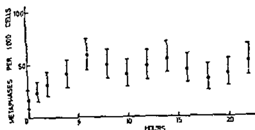


Fig 1 Metaphase per 1 000 cells plotted as a function of time after an intravenous dose of 1 5 mg vincristine to Case 8, with squamous carcinoma of the tongue

cells in mitosis, the number of cells in prophase, the number of cells in metaphase and the number of post-metaphase cells were noted

In one patient the series of biopsies were found to contain buccal mucosal cells which were free from tumour cells from the adjacent carcinoma of the tongue Of these cells, 1 000 were also scored for the mitotic figures mentioned above

Results

The figures given in Table 1 demonstrate the effect of vincristine on mitosis, in this particular instance for a squamous carcinoma of the tongue The figures are fairly representative of those for most of the tumours The metaphase counts (together with bars representing the 95 per cent confidence limits for Poisson statistics on 1 000 cells) are depicted graphically in Fig 1, which gives the number of metaphases per 1 000 cells counted plotted as the ordinate against duration of time after treatment as abscissa

Following the single intravenous dose of 1 5 mg vincristine, there occurred a rapid and apparently linear increase in the metaphase count from a control mean of 16 per 1 000 cells to a maximum of 59 at 6 hours Thereafter the count falls, only to rise again later in a cyclical fashion The mitotic index also increases from a control value of 3 4 per cent to a maximum of 8 4 per cent at 6 hours and as the number of prophases remains relatively constant it must be assumed that the increase in mitoses is due mainly to the increase in the number of cells in metaphase The number of post-metaphase mitoses remains low during the period of metaphase increase and does not increase significantly

The graph depicted in Fig 2 demonstrates the pattern of metaphase accumulation for a squamous carcinoma of the floor of the mouth (Case 2) From this figure it will be seen that for the first hour after the intravenous injection of 1 5 mg vincristine the curve remains flat, and only then does the rise in metaphase count begin This tendency was also apparent in at least two further cases

Table 1

Case 8 Fungating squamous carcinoma, mid third of left side of tongue

Time (h)	No of mitoses per 1 000 cells	No of prophases per 1 000 cells	No of metaphases per 1 000 cells	No of post metaphase figures per 1 000 cells
0	34	14	16 (9–26)	4
1	41	14	23 (15–34)	4
2	50	17	30 (21–44)	2
4	66	11	41 (29–55)	1
6	84	21	59 (45–75)	4
8	75	18	50 (37–65)	7
10	57	9	41 (29–55)	7
12	60	9	48 (35–63)	3
14	69	8	55 (42–71)	6
16	61	10	45 (33–60)	6
18	48	8	36 (25–50)	4
20	50	5	41 (29–55)	4
22	73	14	52 (39–68)	7

At the beginning of each experiment a control biopsy was taken from the patient A dose of vincristine, stored in lyophilized form in ampoules and diluted with saline prior to administration, was then given rapidly intravenously The actual doses administered ranged from 0 5 to 2 mg, depending on the weight and general condition of the patients The total amount of drug given to each patient, decided after consideration of the individual clinical situation, was in every case well below the total doses accepted for the treatment of lymphoma and leukaemia and was therefore not expected to provoke any of the known side effects This indeed proved to be the case, and also in a number of the cases we obtained the impression that there was clinical improvement in the tumour, accompanied by some palliation of symptoms

After the rapid intravenous injection, biopsies were then taken at two-hourly intervals for periods up to 24 hours depending on the clinical state of the patient Because of the nature of the growths, no local anaesthetic was necessary and hence this variable of local interference with the blood supply avoided The biopsies were generally taken from the 'healthy-looking' edge of the tumour, and as far as possible all of them were taken from morphologically similar areas

The biopsies were fixed in Bouin's solution, sectioned at 3 μ and stained with Harris' Alum Haematoxylin and Eosin-Phloxine Every fifth section was mounted and the best section chosen for examination In 1 000 cells the total number of

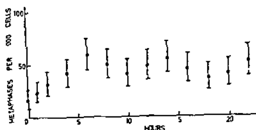


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Table 2

Potential doubling times of human squamous carcinomas as measured by vincristine

Case No	Site and nature of lesion	Dose (mg)	No of metaphases per 1 000 cells at zero time	Mitotic index (%) at zero time	Slope of accumulation curve (metaphases/1 000 cells/h)
1	Fungating supraclavicular mass —anaplastic sq ca? Primary	1 0	27 (18–39)	2 7	2 84
2	Anaplastic sq ca floor of mouth, extending to tongue	1 5	14 (8–23)	2 2	5 82
3	Squamous cervical carcinoma	1 0	20 (12–31)	3 3	9 30
4	Non keratinising sq ca of tongue	0 7	22 (14–33)	4 1	5 61
5	Poorly differentiated, keratinising sq ca of tongue	1 0	11 (5–20)	2 3	3 05
6	Well differentiated, keratinising sq ca of palate	1 0	13 (7–22)	2 8	6 51
7	Sq ca of scalp	1 5	7 (3–14)	1 7	4 95
8	Sq ca of tongue	1 5	16 (9–26)	3 4	6 95
9	Sq ca buccal mucosa and lip	1 0	4 (1 10)	0 9	2 25
10	Cervical sq ca	1 0	9 (4 17)	1 6	3 40
		2 0	14 (8 23)	2 0	4 00
11	Cervical sq ca	0 5	14 (8–23)	2 8	8 60
		1 0	26 (17–38)	3 1	11 30

Table 2 (*cont*)

Potential doubling time T		Time of peak metaphase count (hours post injection)	Duration of linear accumulation (h)	Remarks
Hours	Days			
244.0	10.17	10	0 to 10	1 Possible initial delay 2 Marked trough at 16 hours
119.5	4.98	12	1 to 12	1 Biopsies at 12 and 14 hours unreliable 2 Initial delay
74.85	3.12	8, 14	0 to 8	1 Second peak, but uptake linear only up to 8 hours 2 Marked trough at 18 hours
124.0	5.17	6	0 to 6	Last biopsy at 6 hours so time of peak not reliable
227.25	9.47	6	0 to 6	Last biopsy at 6 hours so time of peak not reliable
106.80	4.45	6	1 to 6	1 Last biopsy at 6 hours so time of peak not reliable 2 Initial delay
140.3	5.85	8, 14	0 to 8	1 Second peak, but uptake only linear up to 8 hours 2 Cyclical variation
100.09	4.17	6, 14	0 to 6	1 Second peak, but not as high as first 2 Cyclical variation
308.04	12.84	8	0 to 8	Biopsies only done at 0, 4 and 8 hours. Time of peak value not reliable and only 2 points on accum curve
204.45	8.52	8	2 to 8	1 Independence of dose tested 2 Initial delay 3 Last biopsies at 8 hours, so time of peak unreliable
173.62	7.23	8	2 to 8	
80.97	3.38	8	0 to 8	1 Independence of dose tested 2 No initial delay 3 Last biopsy at 8 hours, so time of peak unreliable
61.72	2.57	8	0 to 8	

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7	Sq ca of scalp	1 5	7 (3–14)	1 7	4 95
8	Sq ca of tongue	1 5	16 (9–26)	3 4	6 95
9	Sq ca buccal mucosa and lip	1 0	4 (1–10)	0 9	2 25
10	Cervical sq ca	1 0	9 (4 17)	1 6	3 40
		2 0	14 (8–23)	2 0	4 00
11	Cervical sq ca	0 5	14 (8–23)	2 8	8 60
		1 0	26 (17–38)	3 1	11 30

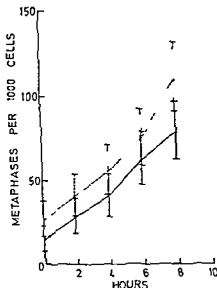


Fig 3 Case 11 with squamous cervical carcinoma Metaphase per 1 000 cells as a function of time after an intravenous dose of 0 5 mg vincristine on Day 1 (—) followed by a further dose of 1 5 mg on Day 3 (— — —) The slopes of the two curves are similar

obtained for the different tumours are given in Table 2, which depicts the comprehensive results for the entire series of experiments

Also included in this table are the periods for which the metaphase accumulation remained linear and the time when the peak value in the metaphase accumulation was recorded

Definite peaks were only recorded in five cases, as in the remaining six cases the serial biopsies had to be stopped before the metaphase accumulation was known to reach its peak The clinical condition of the patient was the deciding factor in terminating the series

It was also important to establish the effect of the magnitude of the dose on the slope of the curves, and if the doses were in any way cumulative We therefore gave one patient (Case 11) 0 5 mg vincristine on day 1 and 1 5 mg on day 3 each dose being followed by serial biopsies up to 8 hours (Fig 3) Similar split doses of 1 and 2 mg respectively were also given to another patient (Case 10) The two regression lines obtained in both cases have been subjected to a statistical comparison In each instance the two lines may be regarded as parallel at the level of $p = 0\ 01$

One particular case was investigated in which it was possible to make a tentative estimate of both the potential doubling time and the clinical doubling time The clinical doubling time of a growing tumour is the observed time taken by the tumour to double its volume, and is obtained from serial caliper measure

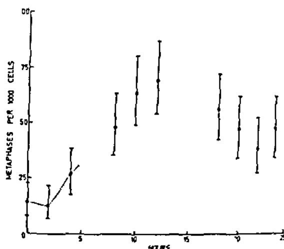


Fig 2 Metaphase accumulation in Case 2 with squamous carcinoma of the floor of the mouth demonstrating an initial lag before the accumulation begins Values at 14 and 16 hours not included as the counts were unreliable The dotted line is included to clarify the early trend of the experimental points

(Cases 6 and 10), but in the remainder the graphs rise linearly from the beginning In the former instances the question arises of a possible delay of up to one hour before the stathmokinetic effect of vincristine becomes apparent, the reasons for which will be discussed later

In view of this variable delay it was decided to omit the values of the metaphase count taken at zero time from the calculations and to determine the accumulation index over the consistently linear portion of the curve only

As stated by STEEL (1968) there are two processes which give rise to a difference between the median cell cycle time and the doubling time of a tumour cell population Assuming that, like the normal tissues from whence they arose, tumours are mixtures of proliferating and non-proliferating cells (MENDELSOHN 1960), one of these differences is the transition of some cells into a non proliferative state The other is the continuous loss of cells from the whole population, and these two processes make the population doubling time longer than the median cell cycle time

The expected doubling time in the assumed absence of cell loss has been called the *potential doubling time*' (STEEL & BENSTED 1965, STEEL) Now, by means of vincristine, cells are blocked and accumulated in metaphase for an accurately determined period (t_a) If t_a is short compared to the population doubling time T, the latter may be calculated from the linear relationship (PUCK & STEFFEN 1963)

$$\ln\ (AI + 1) \approx \ln 2\ \frac{t_a}{T}$$

AI is defined as the arrested index minus the control index The best linear fit to the data is achieved by means of a least-squares analysis The values of T

Table 3

Case 8 Buccal mucosa adjacent to squamous carcinoma

Time (h)	No of mitoses per 1 000 cells	No of metaphases per 1 000 cells	No of post metaphase figures per 1 000 cells
0	24	14 (8–23)	2
1	19	10 (5–18)	1
2	64	47 (35–62)	3
4	90	72 (57–90)	2
6	134	119 (100–141)	2
8	110	98 (80–118)	3
10	114	91 (74–111)	5
12	98	81 (65–100)	6
14	204	162 (140–186)	4
16	74	57 (43–73)	6
18	100	83 (67–102)	7
20	63	36 (25–50)	2
22	77	59 (45–75)	6

on concentration (EVANS et coll 1957, TAYLOR 1965) This conclusion is in accordance with the result obtained by INOUE (1952), who found that the rate of disorganization of an already formed spindle is directly dependent upon the concentration of colchicine used An alternative explanation is that the lag could be produced if the drug acts primarily on the formation of the spindle, in which case the lag relates to the duration of mitosis In plants at least, the lag is also dependent on temperature (EVANS & SAVAGE 1959) and it has also been shown to depend on the mode of administration when used in animal experiments (STEVENS HOOPER 1961)

In the case of vincristine, which is known to abolish the mitotic spindle in oocytes of *Pectinaria* in about 25 minutes at a concentration of 1×10^{4} *mol/l* (MALAWISTA et coll 1968), a similar lag period should be operable We have detected such a lag before accumulation of metaphases by vincristine in three, and possibly in four, of our tumours Unfortunately the procedure is not sufficiently refined to relate the lag period to dose, but the existence of the lag is sufficiently clear to enforce the omission of metaphase counts taken before one hour from the calculations of potential doubling time

FREI III et coll (1964) investigated the stathmokinetic effect of vincristine in a number of situations, namely the root hair of man and mouse, the bone marrow



Fig 4 Case with a rapidly growing painful, non ulcerating squamous carcinoma in which both T and $T_{\frac{1}{2}}$ were estimated

ments of the tumour diameter In the present case only two measurements were made, so that results cannot be accepted with a great degree of confidence Consent was obtained from the patient to take only two biopsies, at 0 and 6 hours, and therefore because of the inherent inaccuracy this case is not included in Table 2 The tumour was a squamous carcinoma of the lip which was painful, rapidly-growing and non-ulcerating (Fig 4) Over the 6 hours the metaphase count per 1 000 cells rose from 6 to 29, giving a potential doubling time of 7 62 days The clinical doubling time from caliper estimations was calculated to be about 10 3 days

In the case of the biopsy taken from buccal epithelium adjacent to a squamous carcinoma (Case 8), the accumulation of metaphases with time is given in Table 3 The curve corresponding to these metaphase counts is given in Fig 5 Although there is no apparent early delay in the metaphase accumulation, the zero value is ignored as in the tumour cases and the regression line has been fitted to the linear accumulation between 1 and 6 hours The slope of the line is 20 34 *metaphase cells/1 000 cells counted/h*, which yields a turnover time of 34 48 hours or 1 44 days The peak of the accumulation occurs at 6 hours, the point at 14 hours being so high as to be regarded as anomalous The mitotic index at zero time for this epithelium is 2 4 per cent and the value of the number of metaphases per 1 000 cells is 14, with a range between 8 and 23 cells

Discussion

Work on colchicine has shown that there exists a latent period from the time of application of colchicine until the drug becomes effective in blocking cells at the metaphase stage The time of this lag period has been shown to be dependent

From the results depicted in Fig 3 we may conclude that the slopes of the metaphase accumulation curves are independent of dose, at least over the range used EVANS et coll (1957) demonstrated this dose independence in the case of colchicine on the root meristem of *Vicia faba*

The values of the potential doubling time derived from the metaphase accumulation curves vary between 2 57 and 12 8 days, with a mean value of 6 3 days However, the maximum value quoted pertained to a patient (Case 9) from whom only three biopsies could be taken, and after the zero value had been omitted, this left only two points from which to derive the slope This value is therefore unreliable and can perhaps with justification be excluded from an estimation of the mean, which then becomes 5 76 days, with an upper limit to the range of 10 17 days

These values may be compared with those obtained by a number of other workers IVERSEN (1967) and REFSUM & BERDAL (1967) used demecolcine to determine the rate of cell proliferation in 61 human tumours For all the cases, their doubling time ranges from 1 0 to 10 8 days, with an average of 2 6 days Although their mean value is lower, this range is comparable with that found in the present work

FREI III and his colleagues used vincristine to determine what they termed the generation time in two human tumours, namely the cutaneous metastases from a breast carcinoma and a lymphosarcoma, for which they derived values of 21 and 29 days respectively Even allowing for the fact that they calculated generation times and not potential doubling times, it is obvious that their times are much longer than those found in the present work As may be seen from Fig 6 however in the case of the breast carcinoma there is an initial delay before there is a rise in mitotic index If, as these authors assumed, the rise in mitotic index is due to accumulated metaphases, the slope of the curve between 2 hours and 6 hours yields a potential doubling time of 6 08 days, which agrees well with our mean value of 5 76 days A least squares fit to the lymphosarcoma curve yields a potential doubling time of about 20 days however and it can only be concluded that the considerable discrepancy in this case is due to the fundamental difference in the nature of the tumour A lymphosarcoma is very different histologically from a carcinoma

More recently SHIRAKAWA et coll (1970) have investigated cell proliferation [illegible] from considerations of the grain count halving time From this they calculated a potential tumour doubling time of 12 days on the basis of a growth fraction of 20 per cent This is *nearer* the upper limit of our range of values

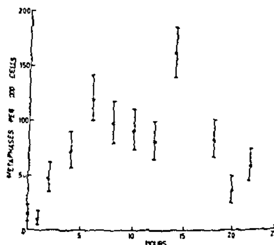


Fig 5 Case 8 The metaphase accumulation in buccal epithelium adjacent to the squamous carcinoma

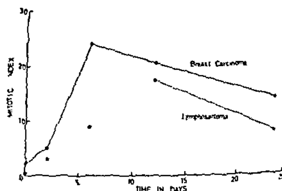


Fig 6 The data of Frei et coll (1964) for mitotic index as a function of time following the administration of vincristine The biopsies were taken from cutaneous metastases in patients with breast carcinoma (—) and lymphosarcoma (— — —) Possible initial lag in the case of the breast carcinoma

of man and cutaneous metastases from a breast carcinoma and a lymphosarcoma A delay time was not commented upon by these authors If, however, the actual values obtained by them in the case of the two tumours are plotted out, the curves shown in Fig 6 are obtained In the case of the breast carcinoma there is a possible initial delay before there is a rise in mitotic index

In the cases where there is no initial delay, it can only be assumed that concentration and absorption *differences lead to a more rapid effect*

The effectiveness of vincristine in holding up the cells at metaphase is very evident in all the present cases Over the period from 1 to at least 6 hours, the number of metaphases is regular In one case examined for 24 hours the metaphase accumulation becomes irregular after 6 hours, but in the other cases the accumulation only becomes irregular at times varying between 8 and 12 hours

alternative procedure becoming available in the near future The present method using vincristine therefore represents a hope along these lines

Acknowledgements

We would like to thank the Medical Superintendent of Groote Schuur Hospital Dr J G Burger for enabling these investigations to take place We also owe a debt of gratitude to Professor Mortimer Mendelsohn, for many valuable discussions whilst on a visit to South Africa

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SUMMARY

The stathmokinetic effect of vincristine is utilized to measure the potential doubling times in a number of human squamous carcinomas A mean value of 5 8 days is obtained and it is shown that this is consistent with the results obtained by other authors

ZUSAMMENFASSUNG

Der stathmokinetische Effekt von Vincristin wurde verwendet um die potentiellen Verdoppelungszeiten bei einer Anzahl von menschlichen Schuppenzell Karzinomen zu bestimmen Es wurde ein mittlerer Wert von 5 8 Tagen erhalten und es wird gezeigt, dass dieser Wert mit den von anderen Autoren erhaltenen Ergebnissen übereinstimmt

RÉSUMÉ

L effet stathmokinetique de la vincristine est utilise pour mesurer les temps de doublement potentiel (PDT) sur un certain nombre de carcinomes epidermoides humains On a trouve une valeur moyenne de 5 8 jours les auteurs montrent que ce resultat concorde avec les resultats obtenus par d autres auteurs

REFERENCES

CARDINALI G CARDINALI GUILIANA and ENEIN M A Studies on the antimitot[illegible]
of [illegible]

CUT[illegible]

EVA[illegible]
col[illegible]

— NE[illegible] The use of colchicine as an [illegible]

The present results also compare favourably with those of TUBIANA et coll. (1969), who investigated five skin carcinomas, and obtained potential doubling times varying between 2 and 5 days (corresponding to actual values of the duration of cell cycle times between one and four days obtained by the method of labelled mitoses and measurement of the growth fraction using MENDELSOHN's method.

FABRIKANT (1970) estimated the duration of the DNA synthesis period in human biopsy specimens of normal and neoplastic cell populations from the larynx, trachea, bronchus and oesophagus of 13 patients by a double-labelling method in vitro. He obtained potential tissue doubling times for malignant tumour cells in the range of 8.95 to 10.86 days.

STEEL derived the following equation for the cell loss factor, ϕ, which is the ratio of the rate of cell loss to the rate of cell production in tumours:

$$\phi = 1 - \frac{T}{T_d}$$

where T is the potential doubling time and T_d is the observed population doubling time. Except for the single case described we have not been in an ethical position to observe clinical doubling times. In this case the value of T is 7.53 days. This is in keeping with the mean obtained from our major series, but when compared with the observed clinical doubling time of 10.3 days, the cell loss factor turns out to be only 27 per cent. This value is low compared to those of other workers (REFSUM & BERDAL, TUBIANA et coll., SHIRAKAWA et coll.), but is in keeping with a tumour which was fast-growing, painful and non-ulcerating, and should perhaps be regarded as the exception and not the rule for squamous carcinomas in general.

The epithelium obtained from buccal mucosa adjacent to a squamous carcinoma (Case 8) cannot of course be regarded as normal, and is histologically hyperplastic. We have been unable to find a value for the potential doubling time of human hyperplastic buccal epithelium in the literature, but it is perhaps interesting to compare our value of 34.5 hours with the value of 42 hours obtained by REISKIN & MENDELSOHN (1964) for the generation time of hyperplastic epithelium from the cheek pouch of the albino hamster. LIPKIN et coll. (1963) observed a cell cycle of about 1 day for rectal epithelial cells, with a potential doubling time which would be somewhat longer.

The use of stathmokinetic methods generally leave much to be desired, and many variables are involved in this type of investigation. However, the method described, which has the advantage of being rapid and simple in execution, gives at least an indication of one tumour-cell parameter. The use of tritiated thymidine is rigorous and complicated, and there seems to be no prospect of a simple

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ÉPITHELIOMAS DE LA RÉGION AMYGDALIENNE

Comparaison entre fractionnement classique et irradiation en deux séries (split-course)

J DUTREIX, M HAYEM, B PIERQUIN, K ZUMMER, C HESSE et A WAMBERSIE

L'objet de cet essai thérapeutique est de comparer les effets obtenus sur la tumeur et les tissus sains par deux fractionnements différents (1) un fractionnement « classique » en 3 seances de 330 rad par semaine, total 4 600 rad en 14 seances, et (2) une irradiation en deux séries qui se compose d'une premiere série concentree, 2 × 850 rad à 48 heures d'intervalle, et d'une seconde série délivrée avec un fractionnement classique, 5 séances de 330 rad (Fig 1)

Les irradiations en deux séries (split-course) sont d'utilisation courante dans le traitement par les radiations et les avantages radiobiologiques que l'on peut en attendre ont ete discutes par différents auteurs (SCANLON 1963, SAMBROOK 1964, HOLSTI 1969) L'originalité du traitement « modifie » en 2 séries que nous avons appliqué dans cet essai, tient a la concentration de la première série en

Soumis à la Redaction le 24 Avril 1973

INOUE S The effect of colchicine on the microscopic and submicroscopic structure of the mitotic spindle *In* The chemistry and physiology of the nucleus Exp Cell Res Supplement *2 (1952), 305*

IVERSEN O H Kinetics of cellular proliferation and cell loss in human carcinomas Europ J Cancer 3 (1967) 389

LIPKIN M, BELL B M and SHERLOCK P Cell proliferation kinetics in the gastrointestinal tract of man I Cell removal in rectum and colon J clin Invest 42 (1963) 767

MALAWISTA S E, SATO H and BENSCH K G Vinblastine and griseofulvin reversibly disrupt the living mitotic spindle Science 160 (1968), 770

MENDELSOHN M L The growth fraction A new concept applied to tumours Science 132 (1960), 1496

PUCK T T and STEFFEN J Life cycle analysis of mammalian cells I A method for localizing metabolic events within the life cycle and its application to the action of colcemid and *sublethal doses of X irradiation Biophys J 3 (1963), 379*

REFSUM S B and BERDAL P Cell loss in malignant tumours in man Europ J Cancer 3 (1967), 235

REISKIN A B and MENDELSOHN M L A comparison of the cell cycle in induced carcinomas and their normal counterpart Cancer Res 24 (1964), 1131

SHIRAKAWA S LUCE J K, TANNOCK I and FREI III E Cell proliferation in human melanoma J clin Invest 46 (b) (1970), 1188

STEEL G G Cell loss from experimental tumours Cell Tissue Kinet 1 (1968), 193

— and BENSTED J P M In vitro studies of cell proliferation in tumours Europ J Cancer 1 (1965) 275

— and LAMERTON L F The growth rate of human tumours Brit J Cancer 20 (1966) 74

STEVENS HOOPER CATHERINE E Use of colchicine for the measurement of mitotic rate in the intestinal epithelium Amer J Anat 108 (1961) 231

TAYLOR E W The mechanism of colchicine inhibition of mitosis I Kinetics of inhibition and the binding of H^3 colchicine J cell Biol 25 (1965), 145

TUBIANA M FRINDEL E and MALAISE E ... ls
in cancer research Normal and malig
Fry M L Griem and W H Kirste
Medical Books London 1969

WEINSTEIN G D and FROST P *Cell proliferation in human basal cell carcinoma* Cancer Res 30 (1970) 727

On pratiquait 3 semaines après, un évidement ganglionnaire Si l'on observait des ganglions métastatiques (N+) avec ou sans rupture de la capsule (C+ ou C—) une irradiation post-opératoire par les photons ou les electrons du Bétatron etait prevue

Les malades ont été répartis en deux groupes recevant respectivement . un fractionnement « classique », 3 × 330 rad par semaine (dose totale 4 600 rad), ou un fractionnement « modifié », 2 × 850 rad à 48 h d'intervalle (dose estimée equivalente a 3 000 rad environ en fractionnement classique), apres 21 jours d interruption, reprise de l'irradiation en fractionnement classique jusqu'à la même dose totale equivalente de 4 600 rad

Tous les malades ont ete traités par les photons de 22 MeV du Bétatron à un debit d environ 60 rad/minute en position assise, par 2 champs latéraux opposés droit et gauche, mesurant en moyenne 6 cm × 8 cm, et à une distance source-peau de 100 cm

La decision d'inclure le malade dans l'essai revenait à un comité formé d oto-laryngologistes et radiotherapeutes Ont éte retenus les malades des 2 sexes, âges de moins de 70 ans, atteints d'un carcinome épidermoide de la région amygdalienne (loge amygdalienne, sillon amygdalien, lesion amygdalovélaire, piliers) et pour lesquels était posée l'indication d'une irradiation par hautes énergies La presence d'adénopathies n'etait pas une cause d'exclusion Par contre, ont été exclus de l essai tous les malades traites anterieurement (par irradiation, chirurgie ou chimiotherapie) et ceux pour lesquels on pouvait prevoir une interruption rapide de l'irradiation

Chaque malade a ete examine une fois par semaine pendant toute la période de l irradiation Une fiche d observation etait remplie à chaque examen clinique La premiere avant le debut du traitement avait pour but de fournir une description detaillee de la tumeur primitive Elle comportait un schéma de la région amygdalienne et des structures avoisinantes, la symptomatologie fonctionnelle et objective, la description des adénopathies homo- et contro-latérales, et l'appréciation du reliquat tumoral en pourcentage par rapport a la description initiale

En raison de la nature subjective des criteres d'appreciation de l effet therapeutique, le malade etait vu par trois medecins qui remplissaient indépendamment les fiches de surveillance L'examen de chaque malade se faisait à l'aveugle dans l ignorance du fractionnement utilisé)

Composition et comparabilité des groupes

Quatre-vingt huit malades vus de 1965 a 1968 on éte inclus dans l'étude Conformément au protocole de l essai, les fractionnements ont été répartis par tirage au sort 48 malades ont reçu un fractionnement classique, 40 un fractionnement modifié

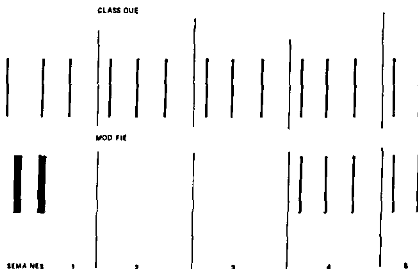


Fig. 1. Fractionnement classique de 14 × 330 rad (en haut) et modifié de 2 × 850 rad + 5 × 330 rad (en bas).

deux séances de 850 rad. Les avantages que l'on peut en espérer ont été discutés ailleurs (Dutreix et coll. 1967), nous en rappelons brièvement les principaux aspects.

On attend de la première série (2 × 850 rad) une réduction importante du nombre de cellules viables et une réduction du volume tumoral plus rapide qu'avec un fractionnement « classique ».

La deuxième partie du traitement (les 5 dernières séances de 330 rad) devrait s'adresser à une population cellulaire peu nombreuse, celle-ci pourrait être plus sensible aux radiations dans la mesure où l'oxygénation (liée à la réduction rapide du volume tumoral) et l'activité mitotique seraient accrues. La deuxième partie du traitement est réalisée en irradiation fractionnée, afin de bénéficier des avantages bien connus du fractionnement : réoxygénation des cellules tumorales hypoxiques entre les séances et repopulation des tissus sains.

Matériel et Methode

A l'Institut Gustave-Roussy, les cancers de l'amygdale étaient traités selon le protocole suivant : (1) 4 500 rad par les photons du Bétatron par 2 champs latéraux droit et gauche opposés, suivi d'une curiethérapie par ^{192}Ir (gouttières vectrices) *délivrant environ 4 000 rad supplémentaires*, (2) s'il existait des ganglions cliniquement palpables ils étaient inclus dans le volume cible du Bétatron.

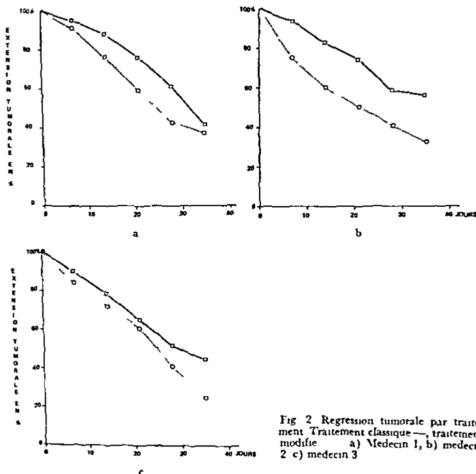


Fig 2 Regression tumorale par traitement Traitement classique —, traitement modifie a) Medecin 1, b) medecin 2 c) medecin 3

Resultats

Regression tumorale

Pour chacun des malades et chacun des observateurs, on a tracé la courbe de regression tumorale en fonction du temps, en prenant comme origine la date du debut du traitement, date a laquelle on considère que l'extension tumorale est egale a 100 pour-cent L'écart theorique de 7 jours entre chaque consultation n a pu être respecté pour chacun des sujets Pour obtenir des points régulièrement espaces, nous avons établi les pourcentages d'extension tumorale correspondant a ces delais reguliers par interpolation linéaire entre les examens réellement effectues Nous avons admis que le pourcentage d extension tumorale varie entre deux

Tableau 1

Description et comparabilité des groupes

	Fractionnement		Comparabilité
	Classique	Modifié	
Nombre de sujets	48	40	
Nombre de femmes	1	4	
Age moyen	57,23	56,61	Non significatif (NS)
Localisation			
Loge amygdalienne	19	16	
Pilier antérieur	10	11	
Pilier postérieur	3	2	
Sillon glosso-amygdalien	4	2	
Voile	2	0	
Autres localisations	9	9	
Côté de la lésion			NS
Droit	27	23	
Gauche	21	17	
Aspect macroscopique dominant			
Végétant	28	25	
Ulcérant	3	2	
Infiltrant	12	9	
Non spécifié	5	4	
Aspect macroscopique multiple	27	26	
Histologie			NS
Epithélioma spino-cellulaire	36	24	
Epithélioma malpighien peu différencié	8	9	
Epithélioma autre ou sans précision	4	7	
T N*			
T_1 T_2	13	17	
T_3 T_4	34	23	NS
N_0	11	10	
N_1 N_2 N_3	36	29	NS

* 1 T non précisé, 2 N non précisés

Dans un premier temps, nous avons comparé ces deux groupes pour les caractères classiques (âge, sexe, T, N, etc.) et pour les caractères décrivant la tumeur primitive (histologie, siège, aspect macroscopique etc.). En aucun cas nous n'avons observé de différence significative (Tableau 1).

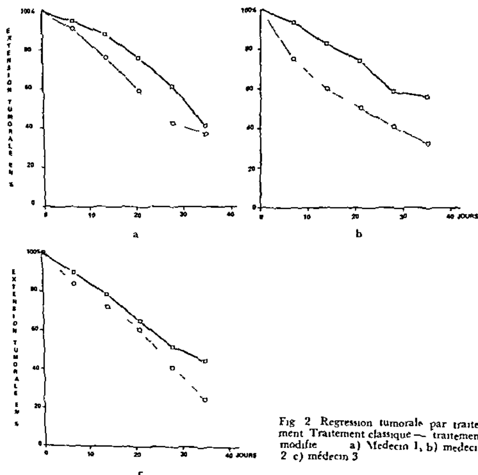


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Histologie			NS
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Epithélioma autre ou sans précision	4	7	
TN*			
T_1 T_2	13	17	
T_3 T_4	34	23	NS
N_0	11	10	
N_1 N_2 N_3	36	29	NS

* 1 T non précisé 2 N non précisés

Dans un premier temps, nous avons comparé ces deux groupes pour les caractères classiques (âge, sexe, T, N, etc.) et pour les caractères décrivant la tumeur primitive (histologie, siège, aspect macroscopique etc.). En aucun cas nous n'avons observé de différence significative (Tableau 1).

Tableau 2

Reliquats tumoraux moyens

Délai	7 jours	14 jours	21 jours	28 jours	35 jours
Fractionnement classique	92,6 (48)	82,6 (48)	71,4 (48)	57,6 (45)	46,1 (32)
Fractionnement modifié	80,9 (34)	68,2 (30)	54,0 (33)	42,1 (35)	31,6 (25)
χ^2	7,0 **	7,84 **	13,43 ***	8,25 **	4,95 *
Signification	p < 1 ‰	p < 1 ‰	p < 1 ‰	p < 1 ‰	p < 5 ‰

L'effectif sur lequel porte le calcul de la moyenne figure entre parenthèses

Pour un petit groupe de sujets, le protocole de traitement prévoyait une irradiation transcutanée seule jusqu'à 8 000 à 8 500 rad (fractionnement classique 18 sujets, fractionnement modifié 11 sujets) Pour ces malades on a bien sûr une courbe allant jusqu'au 56ème jour La faiblesse des effectifs d'une part, les difficultés rencontrées dans l'appréciation du reliquat tumoral au-delà du 35ème jour d'autre part, rendent illusoire toute comparaison des traitements après 5 semaines

Regression ganglionnaire

Pour étudier les adénopathies, nous avons convenu d'observer la régression ganglionnaire relevée pour le plus gros ganglion initial (de l'une quelconque des chaînes jugulo-carotidienne supérieure et moyenne homo- ou contro-latérale)

Le reliquat ganglionnaire à la 5ème semaine a été exprimé en pourcentage de la plus grande dimension initiale du ganglion et regroupé dans les trois classes suivantes régression nulle, reliquat > 75 pour-cent, régression partielle, reliquat de 25 à 75 pour-cent, et régression totale, reliquat < 25 pour-cent

Les résultats des observations des deux médecins pour lesquels nous disposions du plus grand nombre d'examens figurent au Tableau 3 Pour aucun des deux observateurs nous n'avons mis en évidence de différence significative entre les deux fractionnements

Étude des intolérances

Sur chaque fiche d'observation étaient relevés les signes d'intolérance objectifs (reactions cutanées et muqueuses) et subjectifs (dysphagie, douleurs, etc) Nous nous sommes restreints pour l'etude qui suit aux signes objectifs, en raison du manque de fiabilité des autres renseignements

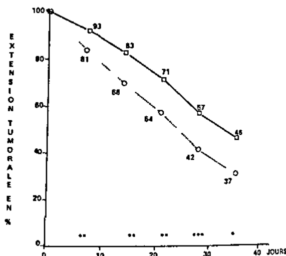


Fig 3 Régression tumorale moyenne Traitement classique —, traitement modifié Signification * $p < 5$ pour-cent, ** $p < 1$ pour cent, *** $p < 1$ pour mille

consultations comme une fonction linéaire du temps, à condition que le délai entre deux observations consécutives d'un même médecin, pour un même malade, n'excède pas 14 jours ou, dans le cas contraire, que la régression tumorale correspondante n'excède pas 20 pour-cent

Comparabilité des observateurs Nous avons tracé pour chaque fractionnement la courbe moyenne des régressions jusqu'au 35eme jour, fournies par chaque observateur, sur les deux groupes de sujets La comparaison de ces résultats a date fixe ne met en évidence aucune différence significative, l'étude de l interaction médecin-fractionnement n'a fait apparaître aucune conclusion significative

Nous avons représenté (Fig 2) pour chaque médecin les résultats de leur observations sur les 2 groupes de sujets L'allure de ces courbes est identique jusqu'au 35eme jour, et l'on retrouve toujours le fractionnement classique situé au-dessus du fractionnement modifié Ces résultats nous ont parus coherents dans leur ensemble et nous avons admis que les observateurs avaient des jugements comparables

Comparaison des regressions tumorales moyennes Pour tracer la courbe de regression tumorale moyenne, relative à chaque traitement, on utilise a chacune des dates fixees la moyenne des points obtenus a cette date pour chacun des observateurs C'est sur ces valeurs que l'on a compare en definitive les resultats moyens obtenus par les fractionnements classique et modifié Des différences significatives apparaissent jusqu'au 35eme jour (Fig 3, Tableau 2) Ainsi, et pendant les quatre premieres semaines de traitement, la regression tumorale est plus rapide pour le fractionnement modifie

Tableau 4

Présence des signes d'intolérance objectifs au cours de la période d'observation

	Fractionnement		Signification
	Classique	Modifié	
Réactions cutanées			
Erythème	100 % (48)	93 % (37)	NS
Pigmentation	98 % (47)	93 % (37)	NS
Desquamation sèche	73 % (35)	53 % (21)	$\chi^2 = 3{,}93$ ($p = 5\,\%$)
Réactions muqueuses (siège)			
Oropharynx	98 % (47)	98 % (39)	NS
Cavité buccale	98 % (47)	98 % (39)	NS
Muqueuse hors faisceau	69 % (32)	80 % (32)	NS

L'effectif sur lequel porte le calcul figure entre parenthèses

d'apparition de chacun de ces signes par un test de rang. La seule différence significative apparaît pour les réactions de la muqueuse qui surviennent plus tôt chez les malades ayant reçu le fractionnement modifié. Le Tableau 5 fournit les délais moyens d'apparition en chaque cas

Comparaison des intensités et des durées des réactions cutanées et muqueuses Les calculs qui suivent se rapportent à chacun des deux médecins qui ont effectué le plus grand nombre d'observations Nous avons étudié pour chaque signe · la durée de la réaction, son intensité maximale, et l'intensité moyenne de la réaction

Tableau 5

Délai moyen d'apparition des intolérances

	Fractionnement		Signification
	Classique	Modifié	
Erythème	16 jours	17 jours	NS
Pigmentation	19 "	18 "	NS
Desquamation sèche	30 "	28 "	NS
Oropharynx	9 "	5 "	$p < 5\,\%$*
Cavité buccale	9 "	5 "	$p < 5\,\%$*
Muqueuse hors faisceau	13 "	9 "	$p < 5\,\%$*

Tableau 3

Reliquats ganglionnaires

Reliquat ganglionnaire	> 75 %	de 25 à 75 %	< 25 %	Total*
Médecin N° 1				
Fractionnement classique	40 % (14)	46 % (16)	14 % (5)	100 % (35)
Fractionnement modifié	24 % (5)	57 % (12)	19 % (4)	100 % (21)
Total	19	28	9	56
χ^2 = 1,54 non significatif				
Médecin N° 3				
Fractionnement classique	56 % (18)	22 % (7)	22 % (7)	100 % (32)
Fractionnement modifié	60 % (12)	15 % (3)	25 % (5)	100 % (20)
Total	30	10	12	52
χ^2 = 0,39 non significatif				

* Le total porte sur l'ensemble des sujets présentant des adénopathies et suivis pendant une période au moins égale à 35 jours

L'effectif sur lequel porte le calcul figure entre parenthèses

Comparaison de la fréquence et des délais d'apparition des signes d'intolérance On a étudié pour chaque traitement le nombre total de sujets qui ont présenté une réaction donnée au cours de la période d'observation et le délai écoulé entre le début du traitement et la 1ère manifestation du signe Pour ces comparaisons, nous avons admis que le malade manifestait une intolérance si l'un quelconque des observateurs en signalait la présence et pris, pour délai d'apparition, le plus court des délais associés à chaque observateur.

Pour chacun des fractionnements figure au Tableau 4 le nombre de sujets qui ont présenté l'une ou l'autre des réactions au cours de la période d'observation Seule, la desquamation sèche semble plus fréquente pour le fractionnement classique que pour le fractionnement modifié.

Sur les Figs 4 et 5 on a porté les histogrammes cumulés des délais d'apparition des intolérances pour les 2 groupes de malades. On a comparé ensuite le délai

Tableau 4

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	Fractionnement		Signification
	Classique	Modifié	
Reactions cutanées			
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Tableau 5

Delai moyen d'apparition des intolerances

	Fractionnement		Signification
	Classique	Modifié	
Erythème	16 jours	17 jours	NS
Pigmentation	19 "	18 "	NS
Desquamation sèche	30 "	28 "	NS
Oropharynx	9 "	5 "	p < 5 %*
Cavité buccale	9 "	5 "	p < 5 %*
Muqueuse hors faisceau	13 "	9 "	p < 5 %*

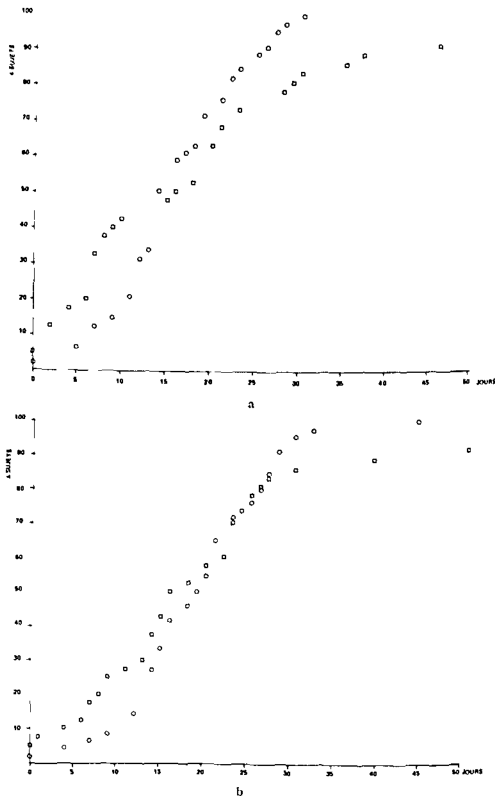


Fig 4 (Pour legende voir la page opposee)

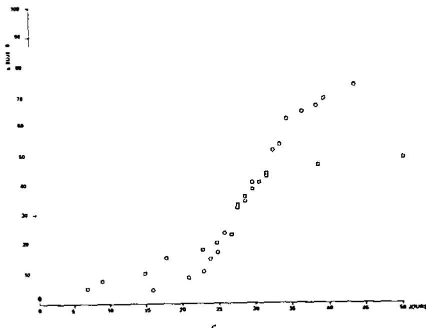


Fig 4 Histogrammes cumulés des délais d apparition des intolérances a) Erytheme, b) pig mentation c) desquamation sèche Traitement classique □ traitement modifié O

pendant la periode au cours de laquelle le signe était présent Malgré une échelle de cotation différente les données des deux observateurs sont concordantes

La comparaison des intensites maximales entre les fractionnements a fait apparaître une différence significative pour les réactions de la muqueuse buccale chez un seul observateur le signe est plus intense pour le traitement modifié

Il nous faut toutefois remarquer que les réactions des muqueuses apparaissent de façon plus tardive pour le traitement classique Nous avons donc étudié cette liaison chez ceux des malades chez lesquels le signe considéré avait disparu avant la fin de la periode d observation Sur les 16 sujets etudiés, la liaison entre le type de fractionnement et l intensité de la reaction disparaît pour cet observateur

Il en est de même pour l'intensité moyenne de chaque signe pendant la période ou ce signe etait présent, sauf dans un cas l'érythème est en moyenne plus élevé dans le fractionnement classique pour l'un des observateurs qui par ailleurs, signalait plus d erytheme tant pour le fractionnement classique que pour le fractionnement modifié

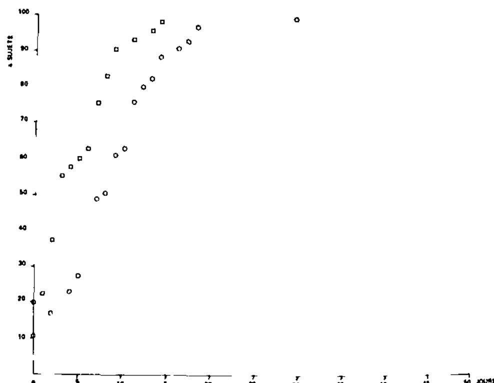


Fig. 5. Histogramme cumulé du délai d'apparition des intolérances. Oropharynx. Traitement classique □ traitement modifié ○

Pour les deux observateurs et les deux fractionnements, la durée de la réaction de la muqueuse de l'oropharynx semble liée à son intensité maximale. Pour une même intensité maximale, la durée de chaque réaction ne diffère pas de façon significative d'un fractionnement à l'autre.

Tableau 6

Traitement consécutif à l'irradiation transcutanée

Traitement	Fractionnement		Total
	Classique	Modifié	
Radiothérapie seule	19	12	31
Radiothérapie + Curie	7	6	13
Radiothérapie + évidement ganglionnaire	3	1	4
Radiothérapie + Curie + évidement ganglionnaire	19	21	40
Total	48	40	88

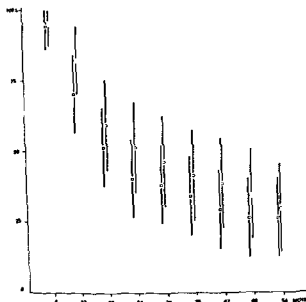


Fig 6 Courbes de survie Traitement classique □, traitement modifié O

Traitements ultérieurs et survie

Traitement consécutif à l'irradiation transcutanée Après l'irradiation transcutanée, on a pratiqué suivant le protocole et tenant compte de chaque malade, un évidement ganglionnaire et une curiethérapie

Le Tableau 6 indique les associations thérapeutiques Il n'y a pas de différence significative entre les 2 groupes de malades

Pour ceux des malades ayant subi un évidement ganglionnaire, nous avons etudie la répartition des ganglions envahis (Tableau 7) De même, les résultats ne sont pas significativement differents

Tableau 7

Envahissement après évidement ganglionnaire

Ganglions	Fractionnement		Total
	Classique	Modifié	
N +	13	15	28
N −	6	6	12
Non précisé	3	1	4

Tableau 8

Taux de survie

Délai (mois)	Fractionnement	
	Classique	Modifié
3	1,00	1,00
6	0,91	0,95
12	0,70	0,82
18	0,51	0,59
24	0,40	0,51
30	0,38	0,46
36	0,34	0,41
42	0,30	0,38
48	0,27	0 35
54	0 27	0,30
60	0,27	0,30

Survie Nous avons calculé par la méthode actuarielle la courbe de survie des deux groupes Le recul minimum de chacun des sujets est de 3 ans La Fig 6 et le Tableau 8 donnent, pour chaque fractionnement, les probabilités de survie jusqu'au 60ème mois Nous n'avons constaté aucune différence significative dans la survie

Nous avons ensuite réparti les sujets en deux groupes en fonction de leur survie afin d'étudier La régression tumorale au 28ème jour (Tableau 9), la distribution du T (Tableau 10) et du N (Tableau 11) en fonction de la survie Pour aucun des deux fractionnements, il n'y a de liaison entre la survie et la régression tumorale au 28ème jour En ce qui concerne le T nos résultats ont confirmé les données de la littérature

Pour les tumeurs avec métastases ganglionnaires nous n'avons mis en évidence sur cet echantillon aucune valeur pronostique de cet envahissement

Discussion

Les principales differences statistiquement significatives entre le fractionnement « classique » et modifié sont une régression plus rapide de la tumeur amygdalienne, une fréquence moindre de la desquamation seche et l'apparition plus precoce des réactions muqueuses pour le fractionnement « modifié »

Régression tumorale

On peut admettre, a partir des données actuelles sur les courbes de survie cellulaire, qu'une dose de 2 × 850 rad entraîne un taux de survie cellulaire très

Tableau 9

Reliquat tumoral moyen au 28ème jour en fonction de la survie

	Survie $\leq$ 38 mois	Survie $>$ 38 mois	Signification
Fractionnement classique	59 8 (30)	53,1 (15)	NS
Fractionnement modifié	46,4 (20)	36 3 (15)	NS

L effectif sur lequel porte le calcul figure entre parenthèses

Tableau 10

Distribution du T en fonction de la survie

Tumeurs	Survie $\leq$ 38 mois	Survie $>$ 38 mois	Total
T1 - T2	13	17	30
T3 T4	43	14	57
Total	56	31	87

χ^2 - 8 83 p $<$ 1 %

Tableau 11

Distribution du N en fonction de la survie

Ganglions	Survie $\leq$ 38 mois	Survie $>$ 38 mois	Total
N0	10	11	21
N1 – N2 – N3	45	20	65
Total	55	31	86

χ^2 = 3 22 non significatif

faible, correspondant a un « volume tumoral » bien inférieur à ce qu'il est habituellement possible d évaluer cliniquement Dans ces conditions, la régression tumorale telle qu'elle a eté observé au cours de cette étude traduit l *élimination* des cellules tuées

Il convient de remarquer que le paramètre « extension tumorale » utilisé dans ce travail, est une évaluation qui n'est pas *nécessairement* une expression objective du volume tumoral elle contient une part d'appréciation subjective de l'observateur Toutefois, cette restriction ne peut mettre en cause les conclusions

de cette étude comparative, comme l'a montré l'analyse de la signification de l'extension tumorale présentée dans un travail antérieur (DUTREIX & WAMBERSIE 1970)

Comme on pouvait s'y attendre, la régression tumorale est plus rapide avec le fractionnement modifié qu'avec l'irradiation classique On peut traduire la différence des courbes en considérant que celle correspondant à l'irradiation classique est schématiquement en retard de 8 jours sur celle que donne l'irradiation concentrée A la troisième semaine (21ème jour) les extensions tumorales sont en moyenne de 54 pour-cent (fractionnement modifié) et 71 pour-cent (fractionnement classique) A la fin de l'irradiation elles sont respectivement égales a 32 et 46 pour-cent

Même si la différence est nette et statistiquement significative le reliquat tumoral reste important dans les deux cas Il est donc douteux que la situation des populations cellulaires (au point de vue oxygénation et taux de prolifération) soit suffisamment différente pour les deux modalités thérapeutiques, au début de la 4eme semaine, pour entraîner une différence de réponse appréciable aux 5 dernières séances, comme on aurait pu l'espérer

Le résultat final ne peut être jugé que de façon globale par le taux de survie des malades celui-ci ne mettant pas en évidence de difference statistiquement significative, les deux types de fractionnement apparaissent donc équivalents en ce qui concerne l'effet sur la tumeur

Effets sur les tissus sains

Reactions cutanées On peut noter pour chacune des trois réactions cutanées étudiées (érythème, pigmentation, desquamation) la similarité des couples de courbes correspondant aux deux types de fractionnement (Fig 4)

Pour le fractionnement modifie l'apparition de la réaction semble plus precoce mais la fréquence finale de la réaction moins elevee (cependant la différence n'est statistiquement significative que pour la frequence de la desquamation) En ce qui concerne ce dernier critere, l'observation montre donc un pourcentage final de desquamation sèche plus faible dans le cas du fractionnement modifié Dans la mesure ou on peut admettre que la desquamation seche correspond a un taux donné de depopulation cellulaire cutanee, ceci suggère que la depopulation est moins profonde pour une irradiation concentree de 2 × 850 rad que pour les 9 séances de 330 rad (ces deux types d'irradiation etant supposés entraîner un même taux de survie cellulaire) si l'on ne tient pas compte de la multiplication cellulaire

Une hypothese plausible est qu'au niveau de la peau, la multiplication cellulaire se trouverait plus activement stimulée après les doses elevées de l'irradiation concentrée

La discussion plus poussée des mécanismes radiobiologiques concernés sort du cadre de ce travail (DUTREIX et coll 1971).

Réactions muqueuses Au niveau de la muqueuse, on note également l'apparition plus précoce des réactions avec le fractionnement modifié La courbe de fréquence cumulée (Fig 5), présente une avance de 4 à 5 jours par rapport à celle du fractionnement classique L'apparition plus précoce de la réaction après l'irradiation concentrée s'explique par le fait que la dose qui doit être atteinte pour provoquer une réaction observable est probablement obtenue à la première séance de 850 rad dans le cas de l'irradiation concentrée tandis qu'avec le fractionnement classique environ 3 séances de 330 rad sont nécessaires

La fréquence des réactions muqueuses atteint 100 pour-cent pour les deux types d'irradiation et on ne peut donc tirer aucune conclusion comparative

En définitive, les deux modalités d'irradiation conduisent au même résultat final exprimé en taux de survie à 5 ans Elles entraînent des réactions muqueuses de même intensité et de même durée, tandis que les réactions cutanées sont moins fréquentes pour le fractionnement « modifié » que pour le fractionnement classique

Il n'apparaît pas d'avantage thérapeutique en faveur de l'une ou l'autre mo-

RÉSUMÉ

Essai thérapeutique comparant sur 88 épithéliomas de l'amygdale un fractionnement classique a une irradiation en 2 séries avec une première série concentrée (2 × 850 rad) Pour le deuxième fractionnement la régression tumorale et les réactions muqueuses sont significativement plus précoces, la fréquence de la desquamation sèche moins élevée Dans les deux groupes la survie à 5 ans l'intensité des intolérances cutanées et muqueuses no sont pas significativement différentes

SUMMARY

In 88 cases of carcinoma of the tonsil a classical fractionation was compared with a split course irradiation the first series of which was concentrated (2 × 850 rad) In those treated with the split course regime tumour regression and mucositis occurred significantly earlier dry desquamation less frequently The 5 year survival rates and the severity of the cutaneous and mucous membrane reactions were not significantly different in the two groups

ZUSAMMENFASSUNG

Bei 88 Fallen mit einem Karzionom der Tonsillen wurde eine klassische Fraktionierung mit einer Split Course Bestrahlung, dessen erste Serie konzentriert worden war (2 × 850 rad) verglichen. Bei den mit dem Split Course' Regime behandelten Fallen setzte der

Tumor Schwund und die Mucositis *signifikant zeitiger ein und die trockene* Abschuppung war weniger haufig Die 5 Jahres Überlebensraten und der Umfang der Reaktionen der Haut und Schleimhaute unterschieden sich in den beiden Gruppen nicht signifikant voneinander

BIBLIOGRAPHIE

DUTREIX J, SCHLIENGER M et al PRIGNEUX M Irradiation en deux séries et irradiation préopératoire en 2 jours J Radiol Électrol 48 (1967), 167

— TUBIANA M, WAMBERSIE A and MALAISE E The influence of cell proliferation in tumours and normal tissues during fractionated radiotherapy Europ J Cancer 7 (1971) 205

— and WAMBERSIE A *Radiobiologic data obtained from clinical observation of the regression* of epithelioma of the tonsil under different fractionation regimes Amer J Roentgenol 108 (1970) 37

HOLSTI L R Clinical experience with split course radiotherapy Radiology 92 (1969) 591

SCANLON P W Split dose radiotherapy follow up in 50 cases Amer J Roentgenol 90 (1963), 280

SAMBROOK D K Split course radiation therapy in malignant tumors Amer J Roentgenol 91 (1964) 37

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BLOOD LYMPHOCYTES AFTER RADIATION THERAPY OF MAMMARY CARCINOMA

H BLOMGREN, ULLA GLAS, B MELEN and J WASSERMAN

It has become increasingly evident that the lymphoid system in human subjects may build up a defence against autochthonous tumours It has been demonstrated that antibodies are produced in some tumour bearing individuals that are directed against certain structures present in the malignant cells (KLEIN et coll 1967, MORTON et coll 1968, EILBER & MORTON 1970, GIRALDO et coll 1971) Moreover, lymphoid cells obtained from subjects with various types of malignant tumours have been held to be able to inhibit in vitro growth of the specific type of tumour cells and such lymphoid cells have also exhibited a more or less specific cytotoxic activity on the former in vitro (HELLSTROM et coll 1968 a, b, BUBENIK et coll 1970, DIEHL et coll 1971, HELLSTROM et coll 1971)

The immune response elicited against a subject's own tumour cells must be considered to be relatively weak and only rarely does the immune defence of the host cause regression of a solid palpable tumour (See EVERSON & COLE 1966) However, it is possible that immunity against a tumour may be of great importance after the mass has been reduced to a minimum by various methods so that the ratio of specifically sensitized lymphocytes to tumour target cells is increased It is therefore desirable that the clinical method used for removing or reducing the tumour cell mass does not interfere negatively with the lymphoid

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cell population of the host Such a negative side effect is well recognized after treatment with various types of cytotoxic drugs (HERSH & OPPENHEIM 1967, CARDOZO 1970), and has also been reported after local radiation therapy of carcinoma (STJERNSWARD et coll 1972) There is reason to assume that immune response against autochthonous tumours which causes destruction of the neoplastic cells or inhibition of their proliferation is mainly via the thymus dependent lymphocyte population (T cells) (MILLER et coll 1964, GRANT et coll 1965) Recent reports have suggested however that the non-thymus processed lymphocytes (B cells) may also, in a specific way, destroy tumour cells (LAMON et coll 1972, O'TOOLE et coll 1973). See ROITT et coll 1969 for review of T and B cells) Attempts have therefore been made to investigate whether the lymphocyte depletion in peripheral blood caused by radiation therapy in carcinoma may be due to a reduction in the number of T or B cells, or both The use of various markers for T and B lymphocytes has suggested that the lymphocyte depletion following irradiation may be mainly due to a diminished number of T cells (STJERNSWARD et coll)

A detailed analysis of the peripheral blood lymphocyte population before and after radiation therapy of carcinoma of the breast is now presented

Materials and Methods Twelve women, aged 52 to 66, with mammary carcinoma were examined The primary diagnosis was obtained by fine needle aspiration biopsy (FRANZÉN & ZAJICEK 1968) The patients are all included in a clinical trial at present proceeding at Radiumhemmet In summary, at the time of diagnosis, patients with operable carcinoma of the breast are randomized into three different groups receiving the following types of treatment (1) radical mastectomy only, (2) radical mastectomy followed by local irradiation, (3) local irradiation followed by radical mastectomy Healthy subjects, matched for age with the affected patients, served as controls

Treatment was given with ^{60}Co gamma irradiation or high energy electrons and the doses were planned individually Postoperative irradiation treatment was directed against the axilla, infra- and supraclavicular regions, both retrosternal regions and the chest wall with preoperative irradiation to the same regions and the breast, the target dose was always 4 500 rad over a period of 6 to 7 weeks

The total number of lymphocytes per μl blood was calculated from conventional counts of the number in a hemocytometer and determinations of the frequency of various cell types on air dried blood smears by May-Grunwald and Giemsa staining

Human lymphocytes which unspecifically bind sheep erythrocytes to their cell membranes (E rosette-forming cells) are considered to be of thymus origin

Lymphocytes having cell surface bound receptors for activated complement C'3 are labelled as nonthymus derived (EAC rosette forming cells) (For references see discussion) The frequency of these cell types in the peripheral blood lymphocyte population was established before and after local radiation therapy in human subjects

Lymphocytes for rosette formation were obtained by gelatin sedimentation from defibrinated blood without heparin, with subsequent filtration through a nylon wool column (PERLMANN & PERLMANN 1970) followed by centrifugation through a layer of ficoll isopaque (BOYUM 1968) The cells prepared in this way regularly contained at least 99 per cent of lymphocytes The preparation of lymphocytes employed for stimulation tests was performed in the same manner except that the filtration through nylon wool was omitted The cells thus contained significantly more monocytes than the cell suspensions used for rosette formation

The experimental procedure for E rosettes (spontaneous rosettes of sheep red blood cells with human lymphocytes) will be described Rosette formation tests were performed by the method described by JONDAL et coll (1972) with certain modifications Sheep red blood cells (SRBC) stored at + 4° C in Alsevers solution were washed three times with tris buffered Hanks balanced salt solution (HTS) and adjusted to a 0 5 per cent suspension (approximately 75 × 10^6 cells/ml) in HTS, 0 25 ml of this suspension being mixed with 1 × 10^6 lymphocytes in 0 25 ml HTS The mixed cells were incubated for 15 min at 37° C, centrifuged at 200 g for 5 minutes in a refrigerated centrifuge (+ 4° C) and incubated overnight at + 4° C The pellet was then gently resuspended and one drop of the cell suspension placed on a glass slide, covered with a cover slip, and sealed, 200 lymphocytes were counted and lymphocytes binding 3 SRBC or more were regarded as positive

The experimental procedure for EAC rosettes (erythrocyte-antibody complement binding cells) was as follows SRBC stored and washed as described above were adjusted to a 5 per cent solution in HTS, 5 ml of this suspension were mixed with an equal volume of a rabbit anti sheep red cell serum diluted 1 2 000 This serum was prepared by injecting SRBC, inactivated before use, into a rabbit The optimal dilution of the serum was determined in preliminary experiments The mixture of anti SRBC serum and SRBC was incubated at 37° C for 1 hour, washed three times with HTS and resuspended in the same volume, 5 ml of active human serum (complement) diluted 1 10 were then added and the mixture incubated again at 37° C After 1 hour the cells were washed three times and adjusted to a 1 per cent suspension, 0 25 ml of which was mixed with 0 25 ml HTS containing 1 × 10^6 lymphocytes The cells were spun at 200 g for 5 min and incubated for 30 min at 37° C, they were then resuspended with the help

Table 1

Number of lymphocytes per µl blood and frequency of EAC and E rosette forming cells before and after preoperative, local radiation therapy of mammary carcinoma in 6 patients

	Mean ± SE	
	Before	After
Lymphocytes per µl	1861 ± 268	880 ± 218 ($0.01 > p > 0.005$)*
EAC cells (%)	20.0 ± 2.7	9.5 ± 1.5 ($p < 0.005$)
E cells (%)	57.7 ± 3.4	60.9 ± 2.6 ($0.3 < p < 0.4$)

* Statistical significance from Student's t test between the values obtained before and after therapy

Table 2

Number of lymphocytes per µl blood and frequency of EAC and E rosette forming cells before and after postoperative local therapy in 6 patients

	Mean ± SE	
	Before	After
Lymphocytes per µl	2001 ± 160	896 ± 221 ($p < 0.005$)*
EAC cells (%)	16.0 ± 2.8	8.3 ± 2.7 ($0.05 > p > 0.025$)
E cells (%)	48.2 ± 4.9	55.2 ± 1.7 ($p = 0.05$)

* Statistical significance from Student's t test between the values obtained before and after therapy

Table 3

Pooled data of Tables 1 and 2

	Mean ± SE	
	Before	After
Lymphocytes per µl	1913 ± 170	880 ± 119 ($p < 0.005$)*
EAC cells (%)	18.0 ± 2.0	9.2 ± 1.5 ($p < 0.005$)
E cells (%)	52.9 ± 3.2	58.4 ± 1.7 ($0.025 > p > 0.01$)

* Statistical significance from Student's t test between values obtained before and after therapy

Table 4

Frequency of EAC and E rosette forming lymphocytes of 6 healthy controls tested on two occasions with a 1 to 3 month interval

	Mean ± SE	
	Test I	Test II
EAC cells (%)	20.0 ± 3.8	21.0 ± 3.9 ($p > 0.4$)*
E cells (%)	56.3 ± 4.1	58.8 ± 2.9 ($p > 0.3$)

* Statistical significance from Student's t test between the two test occasions

of a whirl mixer. The counting of EAC rosettes was carried out as described for E rosettes. Every experiment included a control performed in exactly the same way as stated above except that no complement was added to the incubation mixture.

Lymphocyte stimulation tests were performed by suspensions of lymphocytes being washed three times in HTS by centrifugation, counted in a hemocytometer and suspended in Eagle's medium supplemented with glutamine, penicillin and streptomycin. The medium also contained 15 per cent of heat inactivated human serum. Varying numbers of lymphocytes, suspended in 0.5 ml of this medium, were then pipetted into conical bottomed, 15 ml screw cap tubes. The mitogens, dissolved in the same medium, were then added in a volume of 0.5 ml. Phytohaemagglutinin (PHA, Wellcome) was used in a final concentration of 0.5 μg/ml and poke weed mitogen (PWM, Grand Island Biological Comp., N.Y.) at a final concentration of 0.1 μg/ml. Tubes without mitogens served as controls. The total volume of the incubation mixture was always 1.0 ml and all cell concentrations were set up in duplicate. The tubes were closed loosely with caps and incubated at 37° C in a humified 5 per cent CO_2 atmosphere, after 72 hours 0.1 μCi ^{14}C-thymidine (specific activity 54 mCi/mmol) was added to each tube. (For details concerning culture conditions and measurements of ^{14}C-thymidine uptake, see the preceding paper (GLAS & WASSERMAN 1974).)

Results

The frequency of blood lymphocytes forming E and EAC rosettes following radiation therapy was investigated. Purified blood lymphocytes obtained from 12 individual patients were investigated for the frequency of E and EAC rosette-forming cells before and within four weeks after radiation therapy. Half the patients were treated preoperatively and half of them postoperatively.

Table 1

Number of lymphocytes per µl blood and frequency of EAC and E rosette-forming cells before and after preoperative, local radiation therapy of mammary carcinoma in 6 patients

	Mean ± SE	
	Before	After
Lymphocytes per µl	1861 ± 268	880 ± 218 ($0.01 > p > 0.005$)*
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Number of lymphocytes per µl blood and frequency of EAC and E rosette-forming cells before and after postoperative local therapy in 6 patients

	Mean ± SE	
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E cells (%)	48.2 ± 4.9	55.2 ± 1.7 ($p = 0.05$)

* Statistical significance from Student's t-test between the values obtained before and after therapy

Table 3

Pooled data of Tables 1 and 2

	Mean ± SE	
	Before	After
Lymphocytes per µl	1913 ± 170	886 ± 149 ($p < 0.005$)*
EAC cells (%)	18.0 ± 2.0	9.2 ± 1.5 ($p < 0.005$)
E cells (%)	52.9 ± 3.2	58.4 ± 1.7 ($0.025 > p > 0.01$)

* Statistical significance from Student's t test between values obtained before and after therapy

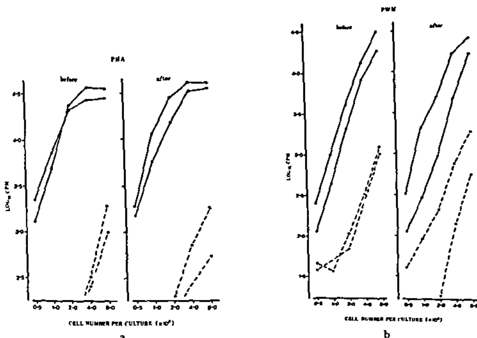


Fig 1 ^{14}C-thymidine incorporations by various numbers of blood lymphocytes obtained before and after therapy in carcinoma and a control The lymphocytes were stimulated by a) PHA and b) PWM O———O Lymphocytes of diseased patient with and O - O without stimulant, ●———● Lymphocytes of control with and ● - ● without stimulant

Fig 1 serves to illustrate a test of the in vitro responsiveness, expressed as $\log_{10}$ counts per minute (cpm) of incorporated ^{14}C-thymidine of lymphoid cells to PHA and PWM as a function of cell number It can be seen that the PHA response increased in an almost linear fashion on increasing the number of cultured cells from $0\ 5 \times 10^{5}$ up to $2\ 0 \times 10$ A further increase in cell concentration resulted in a flattening off of the curves Moreover, this particular test indicated that the PHA responsiveness of the lymphocytes from the patient with carcinoma increased after therapy, assuming that the true reactivity of the control lymphocytes remained at the same level With PWM as a mitogen an almost linear increase in thymidine incorporation was obtained in the dose range of 0 5 to $8\ 0 \times 10$ cells per ml of culture medium It is obvious that in this test the PWM response of the lymphocytes from the patient increased after irradiation

The mitogen responses of the lymphoid cells of the individual patient before and after therapy, are presented in Fig 2 Isotope incorporation, obtained by varying numbers of cultured cells, is expressed as a percentage of cpm incorporated in parallel cultures containing the corresponding number of lymphoid cells

Table 1 indicates that preoperative irradiation significantly reduced the number of lymphoid cells in the blood The proportion of EAC rosette-forming cells diminished significantly whereas the frequency of E-binding cells remained largely unchanged Patients receiving therapy postoperatively (Table 2) also had a significant decrease in lymphocyte counts and in the frequency of EAC rosette-forming cells after irradiation The proportion of 'E cells' in these patients exhibited a slight, but significant increase The pooled data from both groups of patients are presented in Table 3 A closer analysis of the individual tests revealed that the therapy caused a decrease in the number of lymphocytes in all tested patients The frequency of 'EAC cells' decreased in 10 patients, remained constant in 1 and was increased in 1 patient In contrast, the E rosette-forming cells decreased in proportion in 2 patients, remained unchanged in 1 and increased in 9 patients

The lymphocytes of healthy controls, matched for age with the carcinoma patients, were similarly examined with regard to the frequency of EAC and E rosette-forming cells Each was tested on two occasions with an interval of 1 to 3 months Table 4 indicates that the frequency of the two cell types failed to differ significantly between the two tests, nor did they vary to any degree from the values obtained in the carcinoma patients before therapy ($p > 0.3$ for EAC and $p > 0.2$ for E rosette-forming cells)

The mitogen responses of blood lymphocytes following radiation therapy were investigated The results already presented revealed that the therapy reduced the number of blood lymphocytes by approximately 50 per cent and that lymphocytes having receptors for activated C'3 decrease proportionally more than the cells binding SRBC Further to characterize the composition of the lymphocyte pool before and after the therapy, 6 of the patients tested were also examined for the response of their blood lymphocytes to PHA and PWM in vitro

Preliminary tests disclosed that the PHA response of 0.5×10^6 blood lymphocytes, obtained from the same healthy individual, varied considerably on repetitive testing The differences in absolute thymidine incorporations, which exceeded a factor of 3, were considered not to reflect variations in the composition of the lymphocyte population tested, but rather to be due to unknown differences in the culture conditions of the cells For this reason, the mitogen responsiveness of the lymphoid cells from the diseased patients was always expressed in relation to the response of a healthy control whose lymphocytes were cultured at the same time Each carcinoma patient was examined twice, once before and once after treatment Lymphocytes from the same normal donor served as control cells on both these occasions It was thus assumed that the response of the cells from the healthy subjects remained constant before and after the therapy of those with carcinoma

Table 5

PHA responsiveness of various numbers of blood lymphocytes obtained before and after radiation therapy. ^{14}C-thymidine incorporations by the cell cultures are related to the values obtained in corresponding cultures from the controls. Mean values, expressed as percentages of controls calculated from the data presented in Fig. 3

Cell number per culture (x10^5)	Mean ± SE	
	Before	After
0.5	48 ± 14 (0.01 > p > 0.005)*	71 ± 20 (p = 0.2)**
1.0	53 ± 6 (p < 0.005)	70 ± 27 (0.3 > p > 0.2)
2.0	60 ± 12 (0.01 > p > 0.005)	76 ± 23 (0.2 > p > 0.1)
4.0	83 ± 18 (0.2 > p > 0.1)	71 ± 13 (0.2 > p > 0.1)
8.0	89 ± 13 (p > 0.2)	67 ± 15 (0.2 > p > 0.1)

* Statistical significance from Student's t test between the values obtained before therapy and the corresponding values obtained for controls.

** Statistical significance between the values obtained before and after therapy.

Table 6

PWM responsiveness of various numbers of blood lymphocytes obtained before and after radiation therapy. ^{14}C thymidine incorporations by the cell cultures are related to the values obtained in corresponding cultures from the controls. Mean values, expressed as percentages of controls, calculated from the data presented in Fig. 4

Cell number per culture (x10^5)	Mean ± SE	
	Before	After
0.5	65 ± 32 (p > 0.1)*	133 ± 44 (0.05 > p > 0.0025)*
1.0	62 ± 34 (p > 0.1)	214 ± 114 (0.3 > p > 0.2)
2.0	54 ± 30 (0.1 > p > 0.05)	144 ± 80 (p > 0.4)
4.0	49 ± 30 (0.1 > p > 0.05)	93 ± 51 (0.3 > p > 0.2)
8.0	73 ± 27 (p > 0.2)	81 ± 33 (p > 0.4)

* Same as in Table 5.

diseased patients before therapy was significantly lower than that of the healthy controls. This impaired reactivity was best observed with small concentrations of cultured cells. Following irradiation, the responsiveness increased by 0.5 to 2.0 × 10 cells per culture and there was a slight decrease with higher cell numbers. However, neither of these effects, when presented in this manner, was statistically significant. The mean PWM responses of the cell populations mentioned failed to differ significantly from those of the controls.

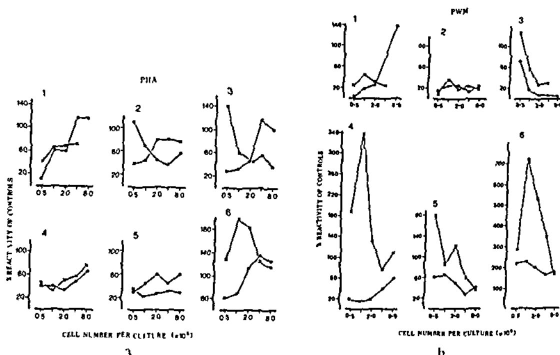


Fig 2 a) PHA and b) PWM responses of the lymphocytes in 6 patients with carcinoma of the breast before and after radiation therapy The cells from each patient were cultivated at five different cell concentrations Symbols represent the thymidine uptakes expressed as percentages of the values for the lymphocytes of the controls Cases 2 3 and 4 received preoperative and Cases 1 5 and 6 postoperative therapy Thymidine incorporations in Case 6 are illustrated in Fig 1 a ● ——● Values obtained before and × —— × after radiation therapy

from the healthy controls The PHA response of the lymphocytes of the affected patients is markedly lower than that of the controls This difference is best observed, *with one exception, with low concentrations of cultured cells* The PHA responsiveness of the cell populations varied following treatment An important observation is that the change in responsiveness was highly dependent on the number of cells per culture Thus, the lymphocytes of the same patient could exhibit an increased PHA reactivity when small numbers of cells, and a decreased reactivity when large numbers of cells, were cultured Similar results were obtained with PWM as a stimulant The responses of the lymphocytes before treatment of the affected patients were markedly lower, with one exception, than those of the controls The PWM response of the lymphocytes following treatment *increased, in 2 patients most markedly This enhanced reactivity* was most conspicuous with low doses of cultured cells

The pooled data, illustrated in Fig 2, are presented in Tables 5 and 6, respectively It is obvious that the PHA responsiveness of the lymphoid cells from the

that, in the human subject, these are T cells (BRAIN et coll 1970, BRAIN & GORDON 1971, JONDAL et coll), (2) The frequency of lymphocytes bearing receptors for activated C'3 (EAC) being considered as non T cells Considerable evidence exists that B cells possess these receptor structures (BIANCO et coll 1970, MICHLMAYR & HUBER 1970, DUKOR et coll 1971, JONDAL et coll) although it is possible that this specific receptor is not entirely restricted to this type of lymphocyte Recent data indicate that another cell type, probably neither a T nor B lymphocyte, designated a 'null lymphocyte' (GREENBERG et coll 1973), also possesses this type of receptor (WIGZELL & PERLMANN, personal communication), (3) Responses of lymphocyte populations in vitro to PHA This mitogen stimulates predominantly T cells as well as lymphoid cells bearing membrane bound γ globulins, presumably B cells (PHILLIPS & ROITT 1973), (4) Responses of lymphocyte populations in vitro to PWM This agent seems to stimulate B cells as wells as T cells (CHESSIN et coll 1966, DOUGLAS et coll 1967) The two former tests identify the different lymphocyte sub populations whereas the latter tend to reflect changes in the relative frequency of cells capable of being triggered to increased DNA synthesis by these mitogens The stimulation tests also revealed the 'health' of the lymphocytes which are potentially capable of blast transformation in response

The results demonstrate that the frequency of E and EAC rosette forming lymphocytes in the patients with carcinoma fails to differ significantly from the values obtained in the controls However, the responsiveness of lymphocytes from the former to PHA proved to be significantly lower than that of cells obtained from controls This difference was most evident when the responses of small numbers of cells per culture were employed, an impaired mitogen responsiveness of such lymphocytes has been confirmed by GARRIOCH et coll (1970), WHITTAKER et coll (1970), DUCOS et coll (1970), HANN & TAKITA (1972), and LANDER & BONE (1973) but denied by ROBINSON & HURVITZ (1966), RICCI et coll (1966), ROBERTS (1970), SUTHERLAND et coll (1971), and FISHER et coll (1972) These discrepancies in the results may be explained by different lymphocyte culture conditions, the relationship between the cell number and the response to the mitogens support this view The importance of an optimal concentration of the stimulant has also been stressed by other investigators (DUCOS et coll , LANDER & BONE) No difference in PWM responsiveness between patients with carcinoma and the normal controls was evident

The irradiation of patients with carcinoma decreased the frequency of EAC rosette-forming lymphocytes significantly whereas the proportion of E rosette-forming cells (mainly T cells) exhibited a slight, but definite increase Such changes were not observed in the controls during the same time period These results are in some disagreement with those obtained by STJERNSWARD et coll

Table 7

Changes of the PHA and PWM responses of the lymphocytes following radiation therapy The values are expressed as percentages of the values, in relation to the controls, obtained before the therapy Mean values calculated from the data presented in Figs 3 and 4

Cell number per culture (x10⁵)	Mean ±SE	
	PHA	PWM
0 5	210±68 (0 025 > p > 0 01)*	333±107 (0 1 > p > 0 05)*
1 0	129±38 (p > 0 2)	532±297 (p > 0 1)
2 0	*125±20 (0 1 > p > 0 05)*	*300± 93 (0 1 > p > 0 05)*
4 0	102±25 (p > 0 4)	258±62 (0 05 > p > 0 025)
8 0	85±19 (p > 0 2)	112± 24 (p > 0 3)

* Statistical significance from Student's t-test between values obtained before (100 %) and after the therapy, with PHA or PWM respectively as stimulants

Enhanced responsiveness after radiation therapy was evident for this mitogen The data are also presented in Table 7 in which the responses of the lymphoid cells after therapy are expressed as percentages of the values obtained before treatment. This manner of presentation demonstrated that the responsiveness to PHA but not to PWM increased significantly following therapy with the lowest concentration of cultured cells

Discussion

The present investigation was undertaken primarily to examine the changes in the peripheral blood lymphocyte population following local therapy of patients with mammary carcinoma Closer knowledge of the side effects on the lymphoid apparatus caused by therapy may be of much importance since the immune defence may be disturbed The aim of the therapy may thus be lost and the patients may be rendered more susceptible to infection

The results of the present work have clearly demonstrated that the lymphocyte pool in peripheral blood is markedly reduced by local therapy, in agreement with the results of other investigators (Goswitz et coll 1963, Meyer & Sayre 1970, McCredie et coll 1972, Stjernsward et coll) In an attempt to characterize any possible changes in the composition of the lymphocyte population, four different markers, more or less specific for various types of lymphocytes were used (1) The frequency of lymphocytes having the capacity to bind SRBC unspecifically to their cell membranes (E cells) Strong support has arisen for the view

that, in the human subject, these are T cells (BRAIN et coll 1970, BRAIN & GORDON 1971, JONDAL et coll), (2) The frequency of lymphocytes bearing receptors for activated C'3 (EAC) being considered as non-T cells Considerable evidence exists that B cells possess these receptor structures (BIANCO et coll 1970, MICHLMAYR & HUBER 1970, DUKOR et coll 1971, JONDAL et coll) although it is possible that this specific receptor is not entirely restricted to this type of lymphocyte Recent data indicate that another cell type, probably neither a T nor B lymphocyte, designated a 'null lymphocyte' (GREENBERG et coll 1973), also possesses this type of receptor (WIGZELL & PERLMANN, personal communication), (3) Responses of lymphocyte populations in vitro to PHA This mitogen stimulates predominantly T cells as well as lymphoid cells bearing membrane bound γ globulins, presumably B cells (PHILLIPS & ROITT 1973), (4) Responses of lymphocyte populations in vitro to PWM This agent seems to stimulate B cells as wells as T cells (CHESSIN et coll 1966, DOUGLAS et coll 1967) The two former tests identify the different lymphocyte sub populations whereas the latter tend to reflect changes in the relative frequency of cells capable of being triggered to increased DNA synthesis by these mitogens The stimulation tests also revealed the health' of the lymphocytes which are potentially capable of blast transformation in response

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who reported the reverse effect after the treatment of patients with mammary carcinoma. However, this apparent discrepancy may be explained by the fact that the frequency of contaminant nonlymphoid cells, such as monocytes, differed in the two investigations. During the present preparation of the lymphocyte suspensions, the cells were passed through a nylon column to remove any others that might be adhering whereas such a step was apparently not included in the tests described by STJERNSWÄRD et coll.

A number of reports have appeared in which the PHA response of lymphocytes from the peripheral blood was examined before and after the therapy of malignancy, some of the investigators reported decreased responsiveness following irradiation (MILLARD 1965, THOMAS et coll. 1971, JENKINS et coll. 1971, STJERNSWÄRD et coll.) whereas others recorded an increase (MCCREDIE et coll. 1972). The present results may offer some explanation for these contradictions. In general, the lymphocytes of the same subject exhibited an increased responsiveness both to PHA and PWM following irradiation when small concentrations of cultured cells were used and a decrease at higher cell concentrations. The results of the statistical analysis however do not permit more than a suggestion that therapy of a malignant lesion may enhance the mitogen responsiveness of lymphocytes and that this effect can only be expressed when small numbers of cells per culture are employed. One explanation for these cell dose dependent results may be that the optimal number of mitogen molecules for triggering the lymphocytes to DNA synthesis is not the same for cells subjected to irradiation. The number of mitogen molecules per cell theoretically available with small numbers of lymphocytes per culture is then higher than when large numbers of cells are used. Thus lymphocytes which require many PHA or PWM molecules to be triggered increase in proportion after therapy either due to enrichment of such cells or to radiation induced changes in the activation threshold of the lymphocytes. Another explanation may be that the nutrients present in the culture medium permit only optimal cell metabolism for a very limited number of lymphocytes, and furthermore that a high cell number per culture, containing the same volume of medium, results in an exhaustion of the nutrients during the first few days of culture and causes subsequent decreased cell activity. This could theoretically create a situation in which small numbers of responsive lymphocytes might yield higher thymidine incorporations per unit cell number on the fourth and fifth days of incubation than larger cell numbers.

In conclusion, the present results have demonstrated that the local therapy of patients with carcinoma of the breast causes a depletion in the pool of lymphocytes in the blood. The frequency of EAC rosette-forming cells decreases and that of E rosette forming cells increases. The lymphocyte stimulation tests with unspecific mitogens suggest an increased reactivity of the lymphocyte population

following irradiation However, this effect is dose dependent and seems to hold true only for low lymphocyte concentrations The increased responsiveness of lymphocytes can be demonstrated only if variations in culture conditions are taken into consideration The relevance of these findings to the in vivo situation remains to be elucidated In any event a large proportion of blood lymphocytes following therapy appear to remain and be capable of being activated and possibly reacting against foreign structures on malignant cells Such an immune response has been suggested to operate in at least some cases (MOORE & FOOT 1949, BERG 1959, CUTLER et coll 1969, HAMLIN 1968, CHAN et coll 1971)

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SUMMARY

Peripheral blood lymphocytes in mammary carcinoma were examined before and after radiation therapy The proportion of those binding sheep erythrocytes (thymus-dependent cells) or those having receptors for activated complement (thymus independent cells) was similar to that of healthy controls The treatment produced lymphopenia and the proportion of thymus independent lymphocytes decreased, although that of the thymus-dependent cells increased The results suggest enhanced mitogen responsiveness with low cell concentrations and impaired reaction of cultures containing those that are h gher

ZUSAMMENFASSUNG

Vor und nach Bestrahlung wurde eine Untersuchung der *Lymphozyten* im peripheren Kreislauf vorgenommen Die Proportion solcher *Lymphozyten*, die Schaferythrozyten zu binden vermochten (thymusabhangige Zellen) und *solcher* die Rezeptoren fur aktivertes Komplement enthielten (thymusunabhangige *Zellen*) war dieselbe wie [illegible] Kontrollen D[illegible]

[illegible] Reaktion auf [illegible]aufweisen, jedoch umgekehrt hohe Zell*konzentra*tionen in Kulturen nur eine geringe Reaktion

RÉSUMÉ

Les auteurs ont examiné avant *et après traitement* par les radiations les lymphocytes du sang périphérique dans le cancer du sein La *proportion* de ceux qui se fixent aux erythrocytes du mouton (cellules thymo-dépendantes) *ou ceux* qui ont des récepteurs pour le com

who reported the reverse effect after the treatment of patients with mammary carcinoma However, this apparent discrepancy may be explained by the fact that the frequency of contaminant nonlymphoid cells, such as monocytes, differed in the two investigations During the present preparation of the lymphocyte suspensions, the cells were passed through a nylon column to remove any others that might be adhering whereas such a step was apparently not included in the tests described by STJERNSWARD et coll

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RÉSUMÉ

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plement active (cellules thymo independantes) sont semblables a celles des sujets temoins sains Le traitement entraine une lymphopenie et la proportion des lymphocytes thymo independants diminue alors que celle des cellules thymo dépendantes augmente Ces resultats font penser que les faibles concentrations cellulaires s accompagnent d une capacite de reponse mitogene augmentee et que les cultures contenant une plus forte concentration cellulaire ont une reaction diminuee

REFERENCES

BERG J W Inflammation and prognosis in breast cancer A search for host resistance Cancer *12 (1959) 714*

BIANCO C, PATRICK R and NUSSENZWEIG V A population of lymphocytes bearing a membrane receptor for antigen antibody complement complexes I Separation and characterization J exp Med 132 (1970) 702

BLOOM H J G and FIELD J R Impact of tumor grade and host resistance and survival of women with breast cancer Cancer 28 (1971), 1580

BOYUM A Isolation of mononuclear cells and granulocytes from human blood Isolation of mononuclear cells by one centrifugation Scand J clin Lab Invest 21 (1968) Suppl 27

BRAIN P and GORDON J Rosette formation by peripheral lymphocytes II Inhibition of the phenomenon Clin exp Immunol 8 (1971) 441

— — and WILLETTS W A Rosette formation by peripheral lymphocytes Clin exp Immunol 8 (1970) 1

BUBENIK J PERLMANN P HELMSTEIN K and MOBERGER G Immune response to urinary bladder tumours in man Int J Cancer 5 (1970) 39

CARDOZO E L The effect of cytostatic therapy with cyclophosphamide alone or in combination with metotrexate on the photohaemagglutinin lymphocyte culture and on the immunoglobulins *Oncology 24 (1970) 261*

CHAN S P MACA R D LEVINE P H and TING R C Immunological studies of human breast cancer I Serum reactivity against a lymphoid cell line (Beles) derived from a breast cancer patient as detected by complement fixation test J nat Cancer Inst 47 (1971) 511

CHESSIN L N BORJESON J WELSH P D DOUGLAS S D and COOPER H L Studies on human peripheral blood lymphocytes in vitro J exp Med 124 (1966) 873

CUTLER S J BLACK M M MORK T HARVEI S and FREEMAN C Further observations on *prognostic factors in cancer of the female breast Cancer 24 (1969) 653*

DIEHL V JEREB B STJERNSWARD J O TOOLE C and ASTROM L Cellular immunity to nephroblastoma Int J Cancer 7 (1971) 277

DOUGLAS S D HOFFMAN P F BORJESON J and CHESSIN L N Studies on human peripheral blood lymphocytes *in vitro* III Fine structural features of lymphocyte transformation by poke weed mitogen J Immunol 98 (1967) 17

DUCOS J MIQUERES J COLOMBIES P KESSONS A and POUJOULET N *Lymphocyte response to* PHA in patients with lung cancer Lancet 1 (1970) 1111

DUKOR P BIANCO C and NUSSENZWEIG V Bone marrow origin of complement receptor lymphocytes Europ J Immunol 1 (1971) 491

EILBER F R and MORTON D L Sarcoma specific antigens Detection by complement fixation with serum from sarcoma patients J nat Cancer Inst 44 (1970) 651

EVERSON T C and COLE W H Spontaneous regression of cancer Saunders Philadelphia 1966

FISHER B SAFFER E A and FISHER E R Studies concerning the regional lymph nodes in cancer Cancer 30 (1972) 120?

FRANZÉN S and ZAJICEK J Aspiration b opsy in diagnosis of palpable lesions of the breast Acta radiol Ther Phys Biol 7 (1968) 241

GARRIOCH D B GOOD R A and GATTI R A Lymphocyte response to PHA in patients with non lymphoid tumours Lancet 1 (1970) 618

GIRALDO G BETH E and HIRSHAUT L Human sarcomas in culture Foci of altered cells and a common antigen induction of foci and antigen in human fibroblast cultures by filtrates J exp Med 133 (1971) 454

GLAS U and WASSERMAN J Effect of radiation treatment on cell mediated immune response in carcinoma of the breast Acta radiol Ther Phys Biol 13 (1974) 83

GOSWITZ F A ANDREWS G A and KNISELEY R M Effects of local irradiation (Co[60]

GR

GREENBERG A H HUDSON L SHEN L and ROITT I M Antibody-dependent cell mediated cytotox city due to a null lymphoid cell Nature New Biology 24? (1973) 111

HAMLIN I M E Possible host resistance in carcinoma of the breast A histological study Brit J Cancer 22 (1968) 34

HANN T and TAKITA H Immunological impairment in bronchogenic carcinoma A study of lymphocyte response to phytohaemagglutinin Cancer N Y 30 (1972) 616

HELLSTROM I HELLSTROM K E PIERCE G E and BILL A H (a) Demonstrat on of cell bound and humoral immunity against neuroblastoma cells Proc nat Acad Sci (Wash) 60 (1968) 1231

— — — and YANG J P S (b) Demonstration of cell bound and humoral immunity to different types of neoplasms Nature (Lond) 220 (1968) 1352

— — and SJOGREN H Demonstration of cell med ated immunity to human neoplasms of various h stological types Int J Cancer 7 (1971) 1

HERS I E M and OPPENHEIM J J Inhibition of *in vitro* lymphocyte transformation during chemotherapy in man Cancer Res 27 (1967) 98

ILBERY P L T RICKINSON A B and THRUM C E Blood lymphocyte repl cating abil ty as a measurement of radiat on dosage Brit J Radiol 44 (1971) 834

JONDAL M HOLM G and WIGZELL H Surface markers on human T and B lymphocytes J exp Med 136 (1972) 207

KLEIN G CLIFFORD P KLEIN E SMITH R T M
CHENAL J H Membrane immunofluo
b opsy specimens and tissue culture J

LAMON E W SKURZAK H M KLEIN E and WIGZELL H *In vitro* cytotoxicity by a non thymus processed lymphocyte population with specificity for a virally determined tumor cell surface antigen J exp Med 136 (1972) 1072

LANDER I and BONE G Lymphocyte transformation in large bowel cancer Brit J Cancer 27 (1973) 409

MCCREDIE J A INCH W R and SUTHERLAND R M Effect of postoperative radiotherapy on peripheral blood lymphocytes in patients with carcinoma of the breast Cancer 29 1972 349

MEYER K K and SAYRE P Rad ation induced lymphocyte immune deficiency Arch Surg 101 (1970 114

MICHLMAYR G and HUBER H Receptor s tes for complement on certain human peripheral blood lymphocytes J Immunol 105 (1970) 670

MILLARD R E Effect of previous irradiation on the transformation of blood lymphocytes J clin Path 18 (1965), 783

MILLER J F A P, TING R C and LAW L W Influence of thymectomy on tumor induction by polioma virus in C57Bl mice Proc Soc exp Biol 116 (1964) 323

MOORE O S and FOOT F W The relatively favorable prognosis of medullary carcinoma of the breast Cancer 2 (1949), 635

MORTON D L, MALMGREN R A, HOLMES E C and KETCHAM A S Demonstration of antibodies against human malignant melanoma by immunofluorescence Surgery 64 (1968), 233

O TOOLE C, PERLMANN P, WIGZELL H, UNSGAARD B and ZETTERLUND C Lymphocyte cytotoxicity in bladder cancer No requirement for thymus-derived cells? Lancet (1973), p 1085

PERLMANN P and PERLMANN H Contactual lysis of antibody coated chicken erythrocytes by purified lymphocytes Cell Immunol 1 (1970), 300

PHILLIPS B and ROITT I M Evidence for transformation of human B lymphocytes by PHA Nature New Biology 241 (1973), 254

RICCI M, PASSALEVA A and RICCA M Mixed lymphocyte reaction in cancer Lancet 2 (1966), 503

ROBERTS M M Lymphocyte transformation in breast cancer Brit J Surg 57 (1970) 381

ROBINSON E and HURWITZ D In vitro studies of lymphocytes from cancer patients Israel J med Sci 2 (1966) 80

ROITT I M, GREAVES M F, TORRIGIANI G, BROSTOFF J and PLAYFAIR J H The cellular basis of immunological responses Lancet 2 (1969) 367

STJERNSWARD J, JONDAL M, VANKY F, WIGZELL H and SEALY R Lymphopenia and change in distribution of human B and T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma Lancet 24 (1972) 1352

SUTHERLAND R M, INCH W R and MCCREDIE J A Phytohemagglutinin (PHA) induced transformation of lymphocytes from patients with cancer Cancer 27 (1971), 574

THOMAS J W, COY P, LEWIS H S and YUEN A The effect of therapeutic irradiation on lymphocyte transformation in lung cancer Cancer 27 (1971), 1046

WHITTAKER M G, REES K and CLARK C G Reduced lymphocyte transformation in breast cancer Lancet 1 (1970) 892

FROM THE DEPARTMENTS OF INTERNAL MEDICINE AND RADIATION THERAPY, UNIVERSITY HOSPITAL, S 221 85 LUND, SWEDEN

AGE VARIATION IN THE UPTAKE OF RADIOIODINE IN THYROTOXICOSIS

G NILSSON and T MOLLER

Measurement of the uptake of radioiodine by the thyroid gland was introduced more than two decades ago as a diagnostic aid This technique is now considered an objective, safe and convenient method of assessing gland activity It has been generally accepted that in the interpretation of the uptake values regard must be paid to the general iodine supply in the region as well as to the possibility of iodine containing drugs, or diagnostic procedures using iodine compounds Another less well known factor influencing the test is the age of the subjects, as observed in euthyroid persons by several authors (PERLMUTTER & RIGGS 1949, MCGREGOR & WAGNER 1958, ODDIE et coll 1960, QUIMBY et coll 1960, BORNER 1961) Reports concerning possible age variation in uptake by toxic goitres are still lacking however, and it was therefore considered of great importance to investigate whether these variations influence the differential diagnosis of thyrotoxicosis

Material and Methods The material consists of 82 hyperthyroid patients (13 men and 69 women, mean age 44), 49 with diffuse goitres (men/women ratio 8 41 mean age 33) and 33 with nodular goitres (men/women ratio 5 28, mean age 60) The hyperthyroid state was invariably confirmed by an elevation of the serum protein bound iodine determined by a Technicon autoanalyser

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MILLARD R E Effect of previous irradiation on the transformation of blood lymphocytes J clin Path 18 (1965), 783

MILLER J F A P, TING R C and LAW I W Influence of thymectomy on tumor induction by polioma virus in C57Bl mice Proc Soc exp Biol 116 (1964) 323

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ROBINSON E and HURWITZ D In vitro studies of lymphocytes from cancer patients Israel J med Sci 2 (1966) 80

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AGE VARIATION IN THE UPTAKE OF RADIOIODINE IN THYROTOXICOSIS

G NILSSON and T MOLLER

Measurement of the uptake of radioiodine by the thyroid gland was introduced more than two decades ago as a diagnostic aid This technique is now considered an objective, safe and convenient method of assessing gland activity It has been generally accepted that in the interpretation of the uptake values regard must be paid to the general iodine supply in the region as well as to the possibility of iodine containing drugs, or diagnostic procedures using iodine compounds Another less well known factor influencing the test is the age of the subjects, as observed in euthyroid persons by several authors (PERLMUTTER & RIGGS 1949, MCGREGOR & WAGNER 1958, ODDIE et coll 1960, QUIMBY et coll 1960, BORNER 1961) Reports concerning possible age variation in uptake by toxic goitres are still lacking however, and it was therefore considered of great importance to investigate whether these variations influence the differential diagnosis of thyrotoxicosis

Material and Methods The material consists of 82 hyperthyroid patients (13 men and 69 women, mean age 44), 49 with diffuse goitres (men/women ratio 8 41, mean age 33) and 33 with nodular goitres (men/women ratio 5 28, mean age 60) The hyperthyroid state was invariably confirmed by an elevation of the serum protein bound iodine determined by a Technicon autoanalyser

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MILLARD R E Effect of previous irradiation on the transformation of blood lymphocytes J clin Path 18 (1965), 783

MILLER J F A P, TING R C and LAW I W Influence of thymectomy on tumor induction by polioma virus in C57Bl mice Proc Soc exp Biol 116 (1964), 323

MOORE O S and FOOT F W The relatively favorable prognosis of medullary carcinoma of the breast Cancer 2 (1949), 635

MORTON D L, MALMGREN R A, HOLMES E C and KETCHAM A S Demonstration of antibodies against human malignant melanoma by immunofluorescence Surgery 64 (1968), 233

O TOOLE C, PERLMANN P, WIGZELL H, UNSGAARD B and ZETTERLUND C Lymphocyte cytotoxicity in bladder cancer No requirement for thymus derived cells? Lancet (1973), p 1085

PERLMANN P and PERLMANN H *Contactual lysis of antibody coated chicken erythrocytes by* purified lymphocytes Cell Immunol 1 (1970), 300

PHILLIPS B and ROITT I M Evidence for transformation of human B lymphocytes by PHA Nature New Biology 241 (1973), 254

RICCI M, PASSALEVA A and RICCA M Mixed lymphocyte reaction in cancer Lancet 2 (1966), 503

ROBERTS M M Lymphocyte transformation in breast cancer Brit J Surg 57 (1970), 381

ROBINSON E and HURWITZ D In vitro studies of lymphocytes from cancer patients Israel J med Sci 2 (1966), 80

ROITT I M, GREAVES M F, TORRIGLANTI G, BROSTOFF J and PLAYFAIR J H The cellular basis of immunological responses Lancet 2 (1969), 367

STJERNSWARD J, JONDAL M, VANKY F, WIGZELL H and SEALY R Lymphopenia and change in distribution of human B and T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma Lancet 24 (1972), 1352

SUTHERLAND R M, INCH W R and McCREDIE J A Phytohaemagglutinin (PHA) induced transformation of lymphocytes from patients with cancer Cancer 27 (1971), 574

THOMAS J W, COY P, LEWIS H S and YUEN A The effect of therapeutic irradiation on lymphocyte transformation in lung cancer Cancer 27 (1971), 1046

WHITTAKER M G REES K and CLARK C G Reduced lymphocyte transformation in breast cancer Lancet 1 (1970), 892

Discussion

The present results provide evidence of a decreased 2 hour uptake with increasing age—a finding which complicates the interpretation of the uptake test in cases with possible thyrotoxicosis As no correlation existed between age and serum protein bound iodine or between age and in vitro triiodothyronine uptake the variability in the severity of thyrotoxicosis cannot explain these findings It seems more likely that the age dependence of 2-hour uptake in hyperthyroid patients is related to the analogous age dependence found in euthyroid subjects (PERLMUTTER & RIGGS, MCGREGOR & WAGNER, ODDIE et coll, QUIMBY et coll, BORNER)

The uptake depends on the absorption rate of iodide in the gastrointestinal tract, the removal rate of iodide from plasma, the iodine fixation in organic form within the gland, and finally on the rate of secretion of hormone iodine of the gland There is evidence that the removal rate of iodide from the plasma diminishes with increasing age (GAFFNEY et coll 1962), but there are no data available of any age variation in the other levels of iodine metabolism

The discrepancy between the negative age correlation of 24 hour uptake found in euthyroid subjects (QUIMBY et coll) and the absence of a similar correlation in hyperthyroid patients found in the present material may be related to the rapid iodine turnover often present in thyrotoxicosis This results in an early maximum of the uptake with high 2-hour values whereas uptake values at 24 hours often are within the normal range despite severe hyperthyroidism

Acknowledgement

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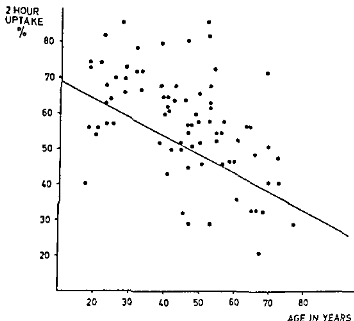
SUMMARY

A negative correlation was found between age and ^{131}I uptake *after 2 hours* but not after 24 hours in a material of 82 hyperthyroid patients, the negative correlation applied in both diffuse and nodular toxic goitres and *is probably related* to the diminished uptake with increasing age previously reported in euthyroid *subjects* The age variation deserves consideration when this technique is used in the differential diagnosis of thyrotoxicosis

ZUSAMMENFASSUNG

Es fand sich eine negative Korrelation zwischen dem Alter und der 131I [illegible]

[illegible] Aufnahme mit steigendem Alter, die fruher bei euthyreoiden Personen beschrieben worden ist zusammen Die altersabhangige Aufnahme muss berucksichtigt werden, wenn diese Technik zur Differentialdiagnose einer Thyreotoxikose verwendet wird



Variation of 2 hour uptake with age in 82 hyperthyroid patients $y = 69.1 - 0.44\ x$, $r = 0.43$, $p < 0.001$

(REILEY & GOCHMAN 1964) and an elevated in vitro triiodothyronine uptake (T_3-test, NOSSLIN 1966) The material includes cases with both diffuse and nodular toxic goitres

Measurements of the uptake in the thyroid gland were performed 2 and 24 hours after oral administration of 15 μCi ^{131}I The detector system consisted of a NaI(Tl) crystal, 4 5 cm $\times$ 5 cm, and a pulse-height analyzer sampling pulses between 300 and 420 keV Background obtained from a measurement over the leg was subtracted A perspex cylinder containing a vial with 15 μCi ^{131}I was used as a standard *The procedure is in accordance with the IAEA recommendations* (IAEA 1962)

In our laboratory, the normal range of uptake is 10 to 35 per cent for 2-hour and 20 to 55 per cent for 24-hour measurements

Results

As evident in the Figure there was an obvious negative correlation ($p < 0.001$) between the 2-hour uptake and the age of the patient This negative correlation was significant for both diffuse goitres ($p < 0.001$) and nodular goitres ($p < 0.01$) On the contrary, no correlation was found between age and 24-hour uptake Likewise, no correlation was found between age and the serum protein bound iodine or in vitro triiodothyronine uptake (T_3-test)

FROM THE DEPARTMENT OF RADIATION THERAPY (DIRECTOR PROF L G LARSSON), UMEÅ UNIVERSITY, S 901 85 UMEÅ, SWEDEN

ACCUMULATION OF COLLIODAL ^{198}Au IN NORMAL BONE MARROW OF RABBITS

Microscopic and autoradiographic investigation

N E SATERBORG

METSCHNIKOFF (1887) observed an ability of some cells to phagocytize foreign particles and bacteria and designated them macrophages or microphages RIBBERT (1904) demonstrated accumulation of a colloidal dye *not only in* macrophages but also in other cells He noticed selective uptake of intravenously injected dye in the endothelium of the capillaries of the bone marrow Cells which accumulated colloidal dyes were brought together by ASCHOFF (1924) to a system denominated 'Das Reticulo-Endotheliale System' LEWIS (1931) and MUDD (1934) ascertained that all cells in the mammalian body possess phagocytic and pinocytic ability The participation of the reticulo-endothelial cells in phagocytosis depends upon the route of administration of particles Injected into the blood they are phagocytized only by cells in direct contact with the circulating blood

Radiation active colloids have been used for in vivo scanning of liver, spleen and lymph nodes and for liver blood flow determinations The accumulation of colloidal ^{198}Au in the bone marrow following intravenous administration to patients with various disorders was investigated by LARSSON & JONSSON (1957),

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RÉSUMÉ

Les auteurs ont trouvé une corrélation negative entre l age et la fixation de ^{131}I après 2 heures mais pas apres 24 heures sur une serie de 82 malades hyperthyroidiens cette correlation negative s applique aussi bien aux goitres toxiques diffus et nodulaires et est probablement liee à la diminution de la fixation quand l age augmente diminution deja signalee chez les sujets euthyroidiens La variation en fonction de l age doit etre prise en considération quand on utilise cette technique pour le diagnostic differentiel de la thyrotoxicose

REFERENCES

Bornfr W Die Radiojodspeicherung der Schilddruse in Abhangigkeit von Alter und Geschlecht Klin Wschr 39 (1961) 1116

Gaffney I E Relationship of age to the thyroidal accumulation renal excretion and distribution of radioiodide in euthyroid man J clin Endocr 22 (1962) 784

International atomic energy agency (I A E A) Calibration and standardization of thyroid radioiodine uptake measurements Recommendations drawn up at a consultants meeting convened by the international atomic energy agency Acta radiol 58 (1962) 233

McGregor G A and Wagner H The influence of age on excretion of radioiodine Lancet II (1958) 612

Nosslin B A simplified technique for the triiodothyronine test (T^3 test) with Sephadex Scand J clin Lab Invest (1966) Suppl No 86 p 177

Oddie T H Thomas I D Rundle F F Myhill J and Catt B Diagnostic limits for thyroidal radioiodine uptake rates J clin Endocr 20 (1960) 389

Perlmutter M and Riggs D S Thyroid collection of radioactive iodide and serum protein bound iodine concentration in senescence in hypothyroidism and in hypopituitarism J clin Endocr 9 (1949) 430

Quimby E H Werner S C and Schmidt C Influence of age sex and season on radioiodine uptake of the human thyroid Proc Soc exp Biol (N Y) 75 (1960) 537

Reilly M and Gochman N A fully automated method for the determination of the serum protein bound iodine Technical Symposium 1964 New York

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ENGSTEDT et coll (1958) and LARSSON et coll (1960, 1964), they found that it was correlated to the condition of the bone marrow In hyperplasia (polycythemia vera, hemolytic disorders) the accumulation was increased and reduced or abolished in destructive bone marrow disorders (myelofibrosis, bone marrow metastases, leukemia) as well as following irradiation (KJELLGREN & JONSSON 1969) Increased accumulation in bone marrow of distal parts of the skeleton was often demonstrated in both hyperplastic and destructive disorders, in the latter cases as a sign of compensatory peripheral hematopoiesis

The intention of the present investigation was to determine the accumulation of colloidal ^{198}Au in the bone marrow at the microscopic level Preliminary results were previously reported by SATERBORG (1971)

Material and Methods

Autoradiography was carried out in 23 male rabbits weighing 2 500 to 4 000 g Colloidal ^{198}Au (AB Atomenergi, Studsvik, Sweden) was processed by the method of DEL TURCO & PIETRA (1960) The delivered sol contained 3 5 mg gold/ml and was diluted 10 times with physiologic saline before injection The specific activity of the diluted sol varied from 1 to 10 mCi/ml As a rule, 5 mCi were injected through the ear vein but in a few rabbits up to 20 mCi After one to two hours the rabbits were anesthetized with mebumal sodium and killed by air insufflation through the ear vein Bone marrow samples were taken after splitting the femur or tibia and fixed in alcohol-formalin (methanol 722 5 g, formaldehyde 85 0 g, acetic acid 42 5 g) for 24 hours Bone marrow puncture of the upper part of the contralateral femur was performed in several anesthetized animals before killing

Preliminary freeze-dried sections of bone marrow were compared with paraffin-embedded sections As no difference was found in the distribution of ^{198}Au only paraffin embedded sections were used in the following experiments The sections were stained with hematoxylin-eosin or with the congo-corinth method of GELLERSTEDT (1944) Bone marrow smears were stained with May-Grunwald Giemsa stain Autoradiography of tissue sections was performed with the stripping film method, of smears with the dipping and stripping film methods and of peripheral blood with the dipping film method (FITZGERALD et coll 1953, ROGERS 1967)

By measuring the activity in sections and smears in a well crystal scintillation counter before and after fixation, it was controlled that only insignificant amounts of the isotope were lost The exposure time was about 3 times the half-life of ^{198}Au (half-life 2 7 days) The exposure took place in a refrigerator at $-20°$ to $-30°$ C A few specimens were stained before autoradiography but as the

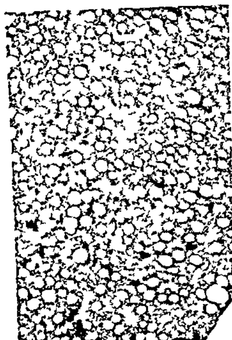


Fig 1



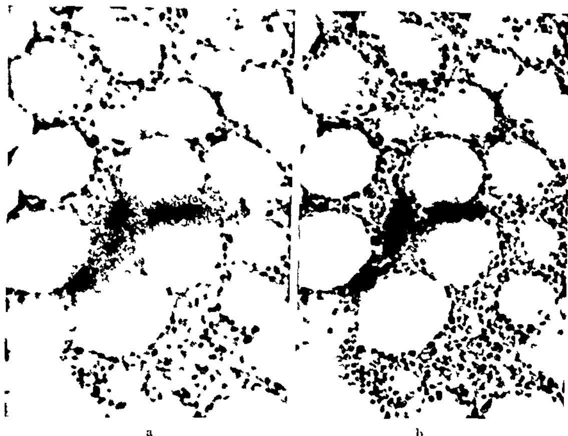
Fig 2

Fig 1 Autoradography of a bone marrow section from femur Spotty distribution of activity Rabbit killed two hours after intravenous injection of 1 0 mCi colloidal ^{198}Au) × 40

Fig 2 Autoradography of a bone marrow section from femur Twenty mCi (20 ml) colloidal ^{198}Au was administered intravenously animal killed after two hours The sinusoids outlined to a longer part The activity concentrated to the sinusoids × 25

stain faded during the photographic processing most of the tissue sections were instead stained through the film The films used were Kodak AR 10 stripping film and Ilford G5 Nuclear Emulsion The films were developed in Kodak D 19 B

Fairly large activities must be administered to obtain satisfactory autoradiograms with the short lived isotope ^{198}Au, this might induce functional and morphologic irradiation changes Twenty mCi was the largest activity injected Provided that 15 per cent of the colloid injected are diffusely distributed in a total bone marrow mass of 150 g and the femur bone marrow is a cylinder of 8 cm length and a diameter of 1 cm the dose delivered to the femur marrow will be in the range of 10 to 50 rad over 2 hours (JOHNS & CUNNINGHAM 1969) Such doses do not influence the morphology and physiology of the bone marrow within 2 hours after administration (FLIEDNER et coll 1955 1961, FLIEDNER &



a b

Fig 3 Autoradiography of a femur bone marrow section (stripping film method) Four μCi ^{198}Au colloid injected intravenously, animal killed after two hours Activity concentrated to a sinusoid, no sign of activity in the surrounding bone marrow The microscope was focused on the film layer in (a) and on the tissue section in (b) ×330

Stodtmeister 1956, Bond et coll 1962, Chone 1964) The activity is mainly concentrated to the reticulo endothelial cells, which, however, are known to be relatively radiation resistant (Shouse et coll 1931, Liebow et coll 1949, Tullis 1949, Chone 1964) In the present material no microscopic signs of radiation injury of the reticulum cells or the surrounding hematopoietic tissue were observed

Microscopic localization of gold colloid Inactive gold colloid containing 3 5 mg gold/ml, processed by the method of Del Turco & Pietra (1960) was administered to four rabbits A large amount of colloid could be injected without interfering with the general condition of the animals, as gold colloid is extremely non toxic (Hahn et coll 1959) In one rabbit 50 ml were administered in one injection but as better outlining of the vessels in the marrow was achieved after repeated injections, 20 ml gold colloid on each of three consecutive days were

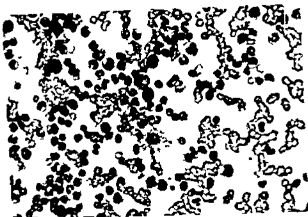


Fig 4 Autoradiography of bone marrow smear from proximal part of femur, 2 mCi colloidal ^{198}Au Activity is registered in reticulum cells no activity corresponding to the cells of the granulocyto- or erythropoiesis × 1 000

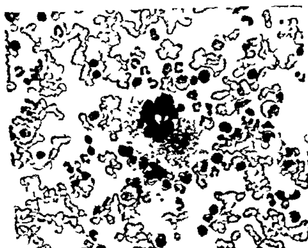


Fig 5 Autoradiography of bone marrow smear from proximal part of femur 2 mCi ^{198}Au colloid Activity was registered corresponding to some megakaryocytes × 1 300

administered to the other three rabbits All the animals were killed during the injection of the last 20 ml colloid

Results

Autoradiography In red bone marrow sections a spotty distribution of activity localized to sinusoids was found when a small amount (1 to 3 ml) of colloidal ^{198}Au had been injected (Fig 1), larger amounts (15 to 20 ml) demonstrated the sinusoids in a longer part (Fig 2) Activity was observed only in the sinusoidal lining cells, no accumulation was found in the hematopoietic cells (Fig 3)

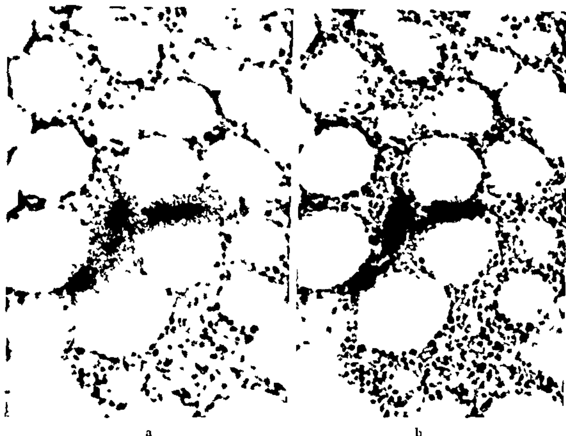


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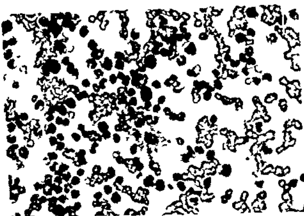


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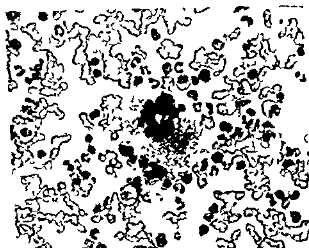


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Fig. 6. a) Tibia bone marrow sample. b) radioautogram after injection of colloidal ¹⁹⁸Au. Gradual decrease of activity from the proximal to the distal end corresponding to the gradual transition from the yellow bone marrow.

The selective accumulation of ¹⁹⁸Au in the sinusoids was confirmed by autoradiography of bone marrow smears, where the activity was traced to a few reticulum cells (Fig. 4). No accumulation of activity was seen in other cells of the bone marrow or peripheral blood in megakaryocytes which sometimes in smears but never in sections contained a large amount of activity (Fig. 5).

In gross specimens exposed on roentgen (Fig. 6) and on autoradiographic (Fig. 7) films it was demonstrated that, as expected, a much larger amount of ¹⁹⁸Au was concentrated in the red than in the fat marrow. The uptake of ¹⁹⁸Au *was, however, not limited to sinusoids of the hematopoietic marrow.* Thus accumulation of activity was also demonstrated in reticulum cells situated in the fat marrow far from hematopoietic cell colonies (Fig. 8). There is a gradual transition from red to yellow marrow in the tibia, and in the same way a gradual transition from a high uptake of colloid in the red hematopoietic marrow to low uptake in the yellow fat marrow was observed (*Fig. 6*). *At microscopy* there was, however, a definite difference between the extension of hematopoietic marrow and that of colloidal ¹⁹⁸Au accumulating sinusoids.

Microscopic localization of gold colloid. Colloidal gold particles are phagocytized in the reticulo-endothelial cells, acting as a selective intravital dye. The capillary vessels in the marrow may thus be visible also in unstained sections



Fig 7 Autoradiography of bone marrow from distal part of tibia In the peripheral hematopoietic marrow more activity is concentrated than in the central fat marrow ×40



Fig 8 Autoradiography of a bone marrow section from the border between red and yellow marrow To the left accumulation of activity in peripheral parts of hematopoietic marrow to the right fat marrow with ^{198}Au colloid accumulated in sinusoids ×100

(Fig 9) If a large number of particles be introduced into the circulation, endothelial cells of larger vessels will also take up some particles (COTRAN 1965) although to a much lower degree than the sinusoidal lining cells (Fig 10)

The fat bone marrow contained three types of cells

(1) Reticulum cells with a large nucleus and regularly a loose chromatin net work and a nucleolus The cytoplasm was very pale in hematoxylin-eosin and congo-corinth stained tissue sections, lacked granules and its outline was difficult to assess This cell type was considered to correspond to the undifferentiated reticular cells of MAXIMOW (1927)

2) Reticulum cells with a medium sized, often oval, nucleus sometimes with a loose, sometimes with a rather dense chromatin net, nucleolus was absent This type of reticulum cells was considered to correspond to the phagocytic reticular



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cells of MAXIMOW, often referred to as fixed macrophages Intermediate forms also occurred

(3) Fat cells usually with a nucleus containing a dense chromatin net The nucleus was often elongated or in cross section deformed due to pressure from stored fat in the cell

Besides the morphologic characteristics the ability to take up colloidal gold could be used for differentiation of the cells in the fat marrow No uptake occurred in morphologically typical undifferentiated reticulum cells or fat cells To distinguish morphologically between undifferentiated reticulum cells (type 1) and phagocytic reticulum cells (type 2) was in some cases difficult The nucleus of a fat cell and a phagocytic reticulum cell could also have a similar microscopic appearance The ability to accumulate colloid then facilitated the identification of the phagocytic reticulum cells The different cell types are illustrated in Fig 11

Discussion

The localization in the red bone marrow of the injected colloid was determined by the number of capillaries patent at the time of injection Phagocytic properties are known to be present in most bone marrow and blood cells (BRANDT 1967) In the present investigation, however, colloidal gold particles accumulated only in the sinusoidal lining cells thus indicating a functionally closed circulation in the marrow Thorotrast injected intravenously in rabbits (ZAMBONI & PEASE 1961) accumulated not only in the sinusoids but also in cells belonging to erythro- and granulocytopoiesis In the present investigation there was no autoradiographic evidence that the blood monocytes or leukocytes took up colloidal ^{198}Au particles The known phagocytic ability of these cells seemed negligible compared to the reticulo-endothelial cells of liver, spleen and bone marrow The megakaryocytes are situated outside the sinusoids in the marrow (HUHN & STICH 1969) and do not normally phagocytize particulate material After a bone marrow puncture, however, the structures of the bone marrow are destroyed and particles in the blood may come into direct contact with the cell membranes of the megakaryocytes These membranes have a strong tendency to attract particles (BEHNKE 1968) and phagocytosis has then been observed in megakaryocytes (ZAMBONI & PEASE 1961) In the intact marrow no accumulation of ^{198}Au colloid was observed in the megakaryocytes in bone marrow sections As the accumulation of colloidal gold in the hematopoietic marrow was strictly confined to the sinusoidal lining cells this may be regarded as a function of the extension of patent sinusoids and the phagocytic ability of the sinusoidal lining cells

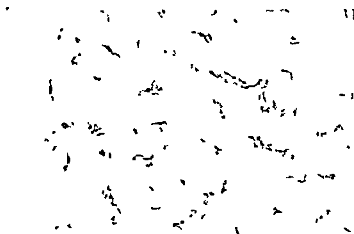


Fig. 9. Unstained bone marrow section from the proximal part of tibia. The sinusoids *outlined by intravenously injected* colloidal gold (20 ml daily on three consecutive days) ×60

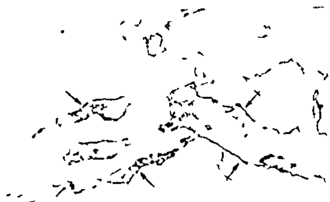


Fig. 10. Unstained bone marrow section from the proximal part of tibia. Accumulation of colloidal gold in the sinusoidal walls (←) and in the endothelium of larger venous vessels (←+) ×100

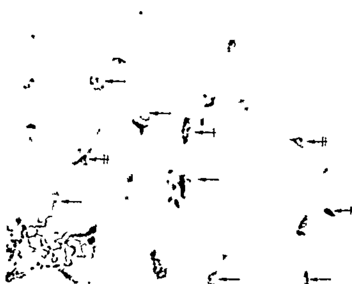


Fig. 11. Cell types in the fat marrow of a normal rabbit. Undifferentiated reticulum cells (←), phagocytic reticulum cells (←+), fat cells (←++). Some of the phagocytic reticulum cells accumulated gold colloid ×410

REFERENCES

ASCHOFF L Das Reticulo-Endotheliale System Ergebn inn Med Kinderheilk 26 (1924), 1

ATKINS H L SCHIFFER L GREENBERG M L and RICHARDS P Reticuloendothelial activity of spleen and marrow studied with ^{99m}Tc J nucl Med 7 (1966), 346

BEHNKE O Electron microscopical observations on the surface coating of human blood platelets J ultrastruct Res 24 (1968) 51

— An electron microscope study of the megakaryocyte of the rat bone marrow I The development of the demarcation membrane system and platelet surface coat J ultrastruct Res 24 (1968) 412

BOND W P FLIEDNER T M and USENIK E Early bone marrow hemorrhage in the irradiated dog Arch Path 73 (1962) 13

BRANDT L Studies on the phagocytic activity of neutrophilic leukocytes Scand J Haemat (1967) Suppl No 2

CHONE B Klinische Strahlenhamatologie *In* Strahlentherapie Sonderband 59 S 190 Herausgegeben von K H Karcher Urban und Schwarzenberg Verlag Munchen Berlin 1964

COTRAN R S Endothelial phagocytosis An electron microscopic study Exp molec Path 4 (1965) 217

DEL TURCO A M and PIETRA R On a method of preparation of colloidal radiogold (^{198}Au) Centro Di Studi Nucleari Di Ispra (CNI) 31 (1960)

VAN DYKE D, SHKURKIN C, PRICE D YANO Y and ANGER H O Differences in distribution of erythropoietic and reticulo-endothelial marrow in hematologic disease Blood 30 (1967), 364

ENGSTEDT L FRANZÉN S JONSSON L and LARSSON L G In vivo localization of colloidal ^{198}Au intravenously injected in polycythemia vera Acta radiol 49 (1958) 66

FITZGERALD P J SIMMEL E WEINSTEIN J and MARTIN C Radioautography Theory, technic and applications Lab Invest 2 (1953) 181

FLIEDNER T M und STODTMEISTER R Zur Knochenmarkwirkung von Radio-Gold (^{198}Au) Strahlentherapie 101 (1956) 289

— SANDKUHLER S und STODTMEISTER R Die Knochenmarkstruktur bei Ratten nach Bestrahlung mit schnellen Elektronen Z Zellforsch 43 (1955), 195

BOND V P and CRONKITE E P Structural cytologic and autoradiographic (H^{3} thymidine) changes in the bone marrow following total body irradiation Amer J Pathol 38 (1961) 599

FOOT N C The endothelial phagocyte A critical review Anat Rec 30 (1925), 15

GELLERSTEDT N Eine neue Methode fur Knochenmarkfärbung an Gewebeschnitten Upsala Lak Foren Forh 49 (1944) 447

GREENBERG M L ATKINS H L and LEWIS S M Erythropoietic and reticuloendothelial function in bone marrow in dogs Science 152 (1966) 526

HAUSER W ATKINS H L and RICHARDS P Five years experience with ^{99m}Tc [illegible]
N Y St J Med 70 [illegible]

HAHN F [illegible]
intr [illegible]
32 ([illegible]

HUHN D and STICH W Fine structure of blood and bone marrow An introduction to electron microscopic hematology J F Lehmanns Verlag Munchen 1969

JOHNS H E and CUNNINGHAM J R The physics of radiology Third edition p 564 Charles C Thomas Springfield Ill 1969

The extension of activity in the bone marrow after intravenous administration of a radiocolloid was primarily thought to register the amount of hematopoietic marrow in an indirect way (ROOT et coll. 1954). This was later confirmed experimentally in normal bone marrow of dogs and rabbits (ATKINS et coll. 1966, GREENBERG et coll. 1966, NELP et coll. 1966). Clinically, however, it was observed that in pathologic conditions differences between the extension of radiocolloid accumulating reticulo-endothelial cells and hematopoiesis may occur (VAN DYKE et coll. 1967, HAUSER et coll. 1970, ROSENTHALL & CHARTRAND 1969). In the present investigation a rough correlation between the extension of hematopoietic bone marrow and uptake of ^{198}Au colloid existed but at a microscopic level a definite discrepancy was observed. This difference may be augmented under pathologic conditions.

SUMMARY

Inactive or radioactive colloidal gold was administered intravenously to rabbits and the distribution in the red and fat bone marrow investigated by microscopy and by autoradiography. The gold colloid was concentrated to the sinusoidal lining cells of the bone marrow. The accumulation was more extensive in the red than in the fat bone marrow. A rough correlation between the hematopoietic activity of the bone marrow and the accumulation of colloidal gold existed but at microscopy a definite difference existed. No hematopoietic cells accumulated colloid.

ZUSAMMENFASSUNG

Inaktives oder radioaktives kolloidales Gold wurde Kaninchen intravenös verabfolgt und dessen Verteilung im roten und fetthaltigen Knochenmark mikroskopisch und autoradiographisch untersucht. Das kolloidale Gold konzentrierte sich in den die Sinus auskleidenden Zellen des Knochenmarks. Die Akkumulation war stärker im roten als im fetthaltigen Knochenmark ausgeprägt. Eine grobe Korrelation zwischen der hämatopoietischen Aktivität des Knochenmarks und der Akkumulation des Goldes lag vor, mikroskopisch bestand jedoch ein klarer Unterschied. Die hämatopoietischen Zellen akkumulierten kein Kolloid.

RÉSUMÉ

De l'or colloïdal inactif ou radioactif a été administré par voie intraveineuse à des lapins et sa distribution dans la moelle osseuse rouge et graisseuse a été étudiée par microscopie et par autoradiographie. L'or colloïdal est concentré dans les cellules qui bordent les sinusoïdes de la moelle osseuse. Son accumulation est plus importante dans la moelle rouge que dans la moelle graisseuse. Il y a une corrélation grossière entre l'activité hématopoïétique de la moelle osseuse et la fixation de l'or colloïdal mais l'examen microscopique montre une nette différence. Les cellules hématopoïétiques ne fixent pas le colloïde.

REFERENCES

ASCHOFF L Das Reticulo Endotheliale System Ergebn inn Med Kinderheilk 26 (1924), 1

ATKINS H L, SCHIFFER L, GREENBERG M L and RICHARDS P Reticuloendothelial activity of spleen and marrow studied with ^{99m}Tc J nucl Med 7 (1966), 346

BEHNKE O Electron microscopical observations on the surface coating of human blood platelets J ultrastruct Res 24 (1968), 51

— An electron microscope study of the megakaryocyte of the rat bone marrow 1 The development of the demarcation membrane system and platelet surface coat J ultrastruct Res 24 (1968) 412

BOND V P, FLIEDNER T M and USENIK E Early bone marrow hemorrhage in the irradiated dog Arch Path 73 (1962) 13

BRANDT L Studies on the phagocytic activity of neutrophilic leukocytes Scand J Haemat (1967) Suppl No 2

CHOVE B Klinische Strahlenhamatologie *In* Strahlentherapie Sonderband 59, S 190 Herausgegeben von K H Karcher Urban und Schwarzenberg Verlag, Munchen, Berlin 1964

COTRAN R S Endothelial phagocytosis An electron microscopic study Exp molec Path 4 (1965) 217

DEL TURCO A M and PETRA R On a method of preparation of colloidal radiogold (^{198}Au) Centro Di Studi Nucleari Di Ispra (CNI) 31 (1960)

VAN DYKE D SHKURKIN C PRICE D, YANO Y and ANGER H O Differences in distribution of erythropoietic and reticulo-endothelial marrow in hematologic disease Blood 30 (1967), 364

ENGSTEDT L FRANZÉN S JONSSON L and LARSSON L G In vivo localization of colloidal ^{198}Au intravenously injected in polycythemia vera Acta radiol 49 (1958), 66

FITZGERALD P J SIMMEL E WEINSTEIN J and MARTIN C Radioautography Theory, technic and applications Lab Invest 2 (1953) 181

FLIEDNER T M und STODTMEISTER R Zur Knochenmarkwirkung von Radio-Gold (^{198}Au) Strahlentherapie 101 (1956) 289

— SANDKUHLER S und STODTMEISTER R Die Knochenmarkstruktur bei Ratten nach Bestrahlung mit schnellen Elektronen Z Zellforsch 43 (1955), 195

— BOND V P and CRONKITE E P Structural cytologic and autoradiographic (H^3 thymidine) changes in the bone marrow following total body irradiation Amer J Pathol 38 (1961) 599

FOOT N C The endothelial phagocyte A critical review Anat Rec 30 (1925), 15

GELLERSTEDT N Eine neue Methode fur Knochenmarkfärbung an Gewebeschnitten Upsala Lak Foren Forh 49 (1944), 447

GREENBERG M L ATKINS H L and LEWIS S M Erythropoietic and reticuloendothelial function in bone marrow in dogs Science 152 (1966) 526

HAUSER W ATKINS H L and RICHARDS P Five years experience with ^{99m}Tc sulfur colloid N Y St J Med 70 (1970) 848

HAHN P F GLEASON G T HERMAN D and FRAZIER H M Hepatic radiography following intravenous administration of colloidal gold under experimental conditions Brit J Radiol 32 (1959) 381

HUHN D and STICH W Fine structure of blood and bone marrow An introduction to electron microscopic hematology J F Lehmanns Verlag Munchen 1969

JOHNS H E and CUNNINGHAM J R The physics of radiology Third edition, p 564 Charles C Thomas Springfield Ill 1969

KJELLGREN O and JONSSON L Bone marrow depression in the pelvis after megavoltage irradiation for ovarian cancer Amer J Obstet Gynec 105 (1969), 849

LARSSON L G and JONSSON L Totalscintigram av manniskokroppen (In Swedish) Svenska Lak -Tidn 54 (1957), 1817

— — Bone marrow scanning after intravenous injection of colloidal gold ^{198}Au *In* Medical radioisotope scanning, IAEA Vienna Vol II p 193, 1964

— ENGSTEDT L , FRANZÉN S and JONSSON L Bone marrow scintigrams An in vivo study of the bone marrow reticulum in malignant bone marrow diseases Acta Un int Cancr 16 (1960), 1473

LEWIS W H Pinocytosis Bull Johns Hopk Hosp 49 (1931) 17

LIEBOW A A WARREN S and DeCOURSEY E Pathology of the atomic bomb casualities Amer J Pathol 25 (1949) 853

MAXIMOW A Lymphoides oder retikulares Gewebe *In* Handbuch der mikroskopischen Anatomie des Menschen Band II, S 335 Herausgegeben von W von Mollendorff J Springer Verlag, Berlin *1927*

METSCHNIKOFF E Sur la lutte des cellules de l organisme contre l invades microbes Ann Inst Pasteur 1 (1887) 321

MUDD S McCUTCHEON M and LUCKÉ B Phagocytosis Phys Rev 14 (1934) 210

NELP W B , LEWIS R J and LARSSON S M Distribution of the erythron and the RES in the bone marrow organ J nucl Med 7 (1966) 366

RIBBERT H Die Abscheidung intravenos injizierten gelosten Karmins in den Geweben Z allg Physiol 4 (1904) 201

ROGERS A W Techniques of autoradiography Elsevier Publ Co New York 1967

ROOT S W GOULD A A KNISELEY R M and TYOR M P The distribution and radiation effects of intravenously administered colloidal ^{198}Au in man Cancer 7 (1954) 856

ROSENTHALL L and CHARTRAND R Radionuclide imaging of the bone marrow Canad med Ass J 100 (1969) 54

SHOUSE S S WARREN S L and WHIPPLE G H Aplasia of marrow and fatal intoxication in dogs produced by roentgen radiation of all bones J exp Med 53 (1931) 421

SÄTERBORG N E Mikrofordelningen av kolloidalt guld i benmargen efter intravenos injektion (In Swedish) Nord Med 85 (1971) 160

TULLIS J L The response of tissue to total body irradiation Amer J Pathol *25 (1949) 829*

ZAMBONI L and PEASE D C The vascular bed of red bone marrow J ultrastruct Res 5 (1961) 65

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COMBINED EFFECT OF ROENTGEN IRRADIATION AND RADIOSTRONTIUM ON THE HAEMATOPOIETIC TISSUES AND THE DEVELOPMENT OF LYMPHOMA IN MICE

BERTIL JARPLID

Radiation from an external roentgen source and internal ^{9}Sr each causes damage to the haematopoietic tissue This is a relatively well known phenomenon and in the long run may involve, inter alia, the risk of leukaemia The external

[illegible] in the skeleton, the radiation will be concentrated to the bone marrow during a lengthy period Leukaemias induced by ^{90}Sr in mice also derive often from the bone marrow (WATANABE 1958, NILSSON 1971), whereas, when induced by fractionated external irradiation, they usually start in the thymus (KAPLAN 1947, JARPLID 1968) It would appear to be a realistic view that in certain situations individuals may be subjected to a combination of these forms of irradiation The effects of such combined irradiation are, however, unknown, which is the reason for the present investigation with respect to changes in the haematopoietic tissue and the occurrence of leukaemia

Submitted for publication 29 August 1973

Table 1

Haematopoietic tissues Survey of experiment

No of animals	Roentgen irradiation	Injection of ^{90}Sr/g body weight	Autopsy, time after last treatment
30 (controls)	—	—	5–97 days
104 (group X)	4 × 140 R	—	4–100 days
104 (group XS)	4 × 140 R	0 2 μCi	4–100 days

Material and Methods

Female CBA mice, aged 30 ± 3 days, were used The total dose of whole body irradiation was given in four equal fractions every fifth day The animals were irradiated in groups of ten in a plastic 'wheel' as described earlier (JARPLID 1968) The roentgen apparatus used, Muller MG 300, was operated at 260 kV, 9 5 mA, filter 0 5 mm Cu + 0 5 mm Al An extra filter of Cu was used with a HVL 1 9 mm Cu at the periphery and a HVL 2 2 mm Cu at the centre The focal distance was 45 cm and the dose rate 74 R/min ^{90}Sr $(NO_3)_2$ was injected intraperitoneally and, in the combined experiments, within five hours after the last fraction of external irradiation For blood examination animals were anaesthetized with ether and blood samples were obtained with a Pasteur pipette from the medial venous plexus of the eye The total numbers of leucocytes were counted in a Burker chamber by conventional manual methods For histologic examination haematopoietic tissues were fixed in Stieve's fluid (ROMEIS 1948), prepared by conventional histologic technique and stained with Ehrlich haematoxylin and eosin

The day for (last) treatment was called day 0

Haematopoietic tissues In this experiment 208 mice were given fractionated irradiation with a total dose of 560 R and were divided into two equal groups (Table 1) From one of these groups (group X) 8 animals were selected at random, subjected to blood examination, and killed at intervals of 4, 8, 11, 17, 21, 24, 28, 32, 39 51, 60, 78, and 100 days after the last fraction The animals in the other group (group XS) were injected in addition with 0 2 μCi ^{90}Sr/g body weight Eight mice from this group were then selected at random, subjected to blood examination and killed at intervals of 4, 8, 12, 15, 20, 25, 29, 35, 40, 50, 57, 76 and 100 days after the injection of ^{90}Sr Thirty mice served as an untreated control group Eight animals from this group were selected at random and handled as above at intervals of 5, 20, 40, 58 and 97 days after the last

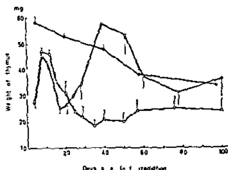


Fig 1 Weight of thymus Untreated controls (●——●) External irradiation, 4 × 140 R (O——O) External irradiation combined with ^{90}Sr, 0 2 μCi/g body weight (□——□) n for each sample = 8 Mean ± SE

irradiation of the X and XS groups At the autopsy the thymic lobes were dissected, weighed separately and fixed for histologic examination The spleen was also weighed and fixed together with femur and sternum

Survival time and lymphoma incidence A survey of different parts of this experiment is given in Table 3 A total of 370 animals was used In the first part (A—C, E) 170 mice were given 4 × 140 R and then injected with ^{90}Sr in various doses (0, 0 1, 0 2 and 0 4 μCi/g body weight) In the second part of the

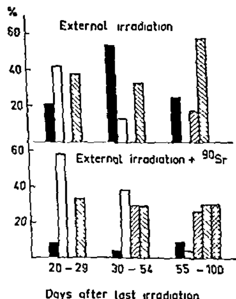


Fig 2 Thymus Incidence of different histologic appearances External irradiation 4 × 140 R and external irradiation combined with ^{90}Sr, 0 2 μCi/g body weight Normal histology ■, Bilateral depletion □, Bilateral regeneration ▨ Histologic asymmetry □ Lymphoma ▩

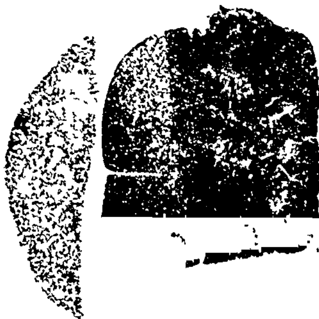


Fig 3 Asymmetric thymus 25 days after combined treatment with external irradiation 4 × 140 R and ^{90}Sr, 0 2 μCi/g body weight Left lobe, lymphocyte depletion with cortical thinning weight 6 0 mg Right lobe regenerated with small medullary areas weight 13 7 mg H & E × 20

experiment 50 mice (F) received 4 × 70 R and another 50 mice (G) 4 × 35 R Then all these hundred animals (F, G) were injected with 0 2 μCi ^{90}Sr/g body weight Fifty mice (H) were not irradiated but injected with 0 2 μCi ^{90}Sr/g body weight at an age of 45 ± 3 days, which corresponds to the age of the irradiated mice at the end of irradiation Another 50 mice (D) were thymectomized 5 to 7 days before the start of irradiation at about 30 days of age These animals received 4 × 140 R and then 0 2 μCi ^{90}Sr/g body weight Thymectomy was performed according to the method described by SJODIN et coll (1963)

All animals were autopsied as soon as possible after their natural death To get fresh specimens for haematology and histology some animals were killed in a moribund state The weight of thymus and spleen was noted and specimens from thymus, spleen, external lymph nodes, sternum and femur were fixed for histologic examination

Results

Haematopoietic tissues

Thymus The changes in total weight of the thymus after fractionated irradiation (group X) and after additional injection of ^{90}Sr (group XS) are illustrated in Fig 1 In both groups the first phase of regeneration had started by day 4 Immature lymphoid cells with a lymphoblast-like appearance predominated in the thymic cortex At the maximum of this regeneration phase on days 8 to 12

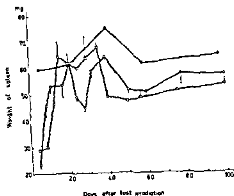


Fig 4 Weight of spleen Untreated controls (●———●) External irradiation 4×140 R (O———O) External irradiation combined with ^{90}Sr 0 2 μCi/g body weight (□———□) n for each sample = 8 Mean ± S E

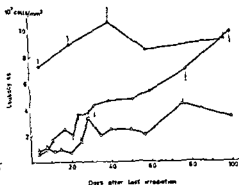


Fig 5 Total number of leukocytes in peripheral blood Untreated controls (●———●) External irradiation, 4×140 R (O———O) External irradiation combined with ^{90}Sr 0 2 μCi/g body weight (□———□) n for each sample = 8 Mean ± S E

the histologic appearance of the thymus was almost normal In both groups the thymus thereafter again decreased in weight as a result of a new and bilateral lymphocyte depletion (second phase of lymphocyte depletion, JÄRPLID 1968) in the thymic cortex, which thereby acquired an irregular, thin and less dense appearance (cortical thinning, Fig 3, left lobe)

In the period 20 to 29 days the thymus in the XS group was characterized by a high incidence (58 per cent) of bilateral lymphocyte depletion, which resulted in a further decrease in weight of the organ (Figs 1, 2) In the X group a second phase of regeneration set in with a new increase in weight and a higher incidence of histologically normal thymus than in the XS group (21 and 8 per cent respectively) The incidence of histologic asymmetry (i e one lobe had a normal histologic appearance or was in a regeneration phase, while the other lobe was the site of depletion of different degrees, Fig 3, JARPLID 1968) was about the same in the two groups (33 to 38 per cent) No case of bilateral symmetrical regeneration was seen during this period

During the period 30 to 54 days the thymus of the X group had its maximum in weight and in incidence of normal histology (54 per cent, Figs 1, 2) The incidence of bilateral lymphocyte depletion was low (13 per cent) In the XS group the mean weight of the thymus had its minimum in this period In 67 per cent of the animals the thymus exhibited morphologically bilateral lymphocyte depletion or regeneration The incidence of normal thymus was very low (4 per cent)

Table 2

Individual cases of lymphoma in mice killed at monthly intervals (n for each sample = 8) after combined treatment with fractionated irradiation (4 × 140 R) and ^{90}Sr (0.2 μCi/g body weight)

Time after last irradiation (days)	Total number of leukocytes/mm³ blood	Haematopoietic tissues involved		
		Thymus	*Bone marrow*	*Spleen*
57	900	+	−	−
76	3 900	+	+	+
76	1 400	+	−	−
100	39 800	+	+	+
100	2 600	+	−	−
100	3 700	+	−	−
100	4 900	+	+	+
100	3 100	+	−	−

During the last period investigated (55 to 100 days) the thymus in the X group had a relatively normal mean weight though the incidence of normal thymus had decreased again (Figs. 1, 2). Instead in 54 per cent of the animals the thymus was asymmetrical mainly with one normal lobe and one lobe in regeneration. No case of lymphoma appeared in this group.

In the XS group the thymic weight was rather low. Histologically three thymic appearances predominated, bilateral regeneration (26 per cent), asymmetry (30 per cent) and lymphoma (30 per cent). In only 9 per cent of cases was the thymus histologically normal.

Spleen. The changes in weight of the spleen are illustrated in Fig. 4. Both in groups X and XS the histologic appearance of the red pulp was initially characterized by a moderately increased extramedullary haematopoiesis. This compensatory haematopoiesis persisted for about three weeks in the X group and *during the entire observation period in the XS group.* Cells from both the erythroid, myeloid and the megakaryocytic cell series successively replaced the initially predominating red cell precursors in this haematopoiesis.

In both groups the number of lymphocytic cells decreased in the periphery of the spleen follicles. In this region accumulations of larger lymphoid cells (germinal centres) appeared after about three weeks in the X group and about two months in the XS group.

Bone marrow. The acute radiation injury was histologically characterized by dilatation of sinusoids, haemorrhage and reduced cellularity. The degree of these



Fig 6 Thymus One hundred days after combined treatment with external irradiation 4 × 140 R and ⁹⁰Sr 0 2 μCi/g body weight Unilateral lymphoma of right lobe weight 28 3 mg Left lobe normal weight 10 4 mg H & E × 20

changes varied between different sections of the marrow from a moderate degree of cellular depletion to large haemorrhages with only a few scattered haematopoietic cells During this cellular depletion period, cells belonging to the granulocytic series predominated in the marrow The injury was most severe during the period 4 to 8 days after external irradiation and 4 to 20 days after combined treatment In the X group focal regeneration led to a relatively normal histologic appearance by day 17 In the XS group, however, the regeneration was delayed and a successively increased cellularity during the period 20—29 days led to a relatively normal histology of the marrow by days 35, 40 and 50 Thereafter, however, and during the rest of the observation period, the cellularity of the bone marrow of the femur was again moderately decreased at the same time as the sternal bone marrow remained relatively normal

Peripheral blood After external irradiation the total number of leucocytes increased successively from a minimum at day 4 to a normal mean value at day 100 In the group treated with combined external and internal radiation, however, the number of leucocytes was below normal during the whole observation period (Fig 5)

Development and incidence of lymphoma

In mice which were killed periodically 4 to 100 days after treatment

Table 3

Incidence of lymphoma and latency time for lymphoma development

No of mice		Dose (R)	Dose of ^{90}Sr (μCi/g body weight)	Incidence of lymphoma, per cent			Mean latency time (days) ± SE	
				Thymic	Non thymic	Total	Thymic	Non thymic
A	48	4×140	—	37	17	54	210±11	233±33
B	48	4×140	0.1	60	13	73	177± 9	199±17
C	46	4×140	0.2	74	13	87	164± 9	179±17
D	50	4×140 (th x)*	0.2	—	28	28	—	191±15
E	28	4×140	0.4	68	3	71	184±16	134
F	50	4× 70	0.2	46	16	62	255±27	257±31
G	50	4× 35	0.2	18	32	50	279±40	275±22
H	50	—	0.2	2	12	14	181	278±51
	370							

* th x = thymectomy

type, which is usually seen after fractionated leukaemogenic irradiation (KAPLAN 1947, JARPLID 1968). Of 5 cases localized only to the thymus 3 were unilateral (Fig. 6). Three cases were generalized with lymphoma changes also in bone marrow and spleen. Seven out of eight mice with lymphoma changes had a low or rather low number of leukocytes in the peripheral blood.

In those groups of the animals which were used for determining survival time and tumour incidence, the cases of lymphoma were divided into two main groups according to the localization of the predominating changes, thymic lymphomas and non-thymic lymphomas.

Thymic lymphoma. The thymus was generally much enlarged (mean weight 407 ± 23 mg) and the two lobes were often indistinguishable as a result of accretion. In some cases the changes were localized only to the thymus. Often, however, extra-thymic haematopoietic tissues were also affected. Tumour cells were then predominatly localized to the red pulp of the spleen, with or without enlargement of this organ, and to the bone marrow and lymph nodes (generalized thymic lymphoma).

Non-thymic lymphoma. Lymphoma appeared in extra thymic haematopoietic tissues with slight or no enlargement of the thymus (mean weight 23 ± 3 mg). The spleen, the lymph nodes or both were generally the site of lymphoma

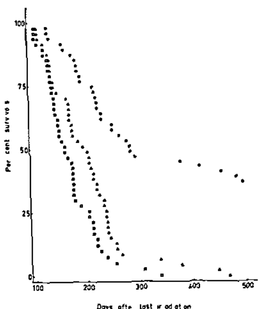


Fig 7 Survival time for mice External irradiation 4 × 140 R (O——O) External irradiation combined with ^{90}Sr, 0 1 μCi/g (Δ——Δ) and 0 2 μCi/g body weight (□——□)

changes and often enlarged, and a heavy proliferation of lymphoid tumour cells was seen in the bone marrow In the case of enlargement of the thymus the lymphoma changes were primarily localized to the peripheral parts of the thymic lobes, giving the impression of being metastases

Lymphomas which were localized around the vertebral column, predominantly around the lumbar vertebrae, were seen after treatment with reduced doses of external irradiation and 0 2 μCi ^{90}Sr/g body weight These tumours often reached a size of a pea to a hazelnut They seemed to develop from the bone marrow of the vertebrae and they sometimes infiltrated the vertebral canal but dit not affect the bone tissue proper

Incidence of lymphoma The incidence of lymphoma in different parts of the experiment is shown in Table 3 The spontaneous incidence of lymphoma in this strain is about one per cent (NILSSON 1971) After treatment with 0 2 μCi ^{90}Sr/g body weight thymic lymphoma appeared in 2 per cent of animals and non-thymic lymphoma in 12 per cent In the group which received 4 doses of 140 R and various doses of ^{90}Sr the incidence of thymic lymphoma after only fractionated irradiation (37 per cent) was doubled after additional treatment with 0 2 μCi ^{90}Sr/g body weight (74 per cent) An addition of 0 1 μCi ^{90}Sr resulted in 60 per cent lymphoma and an addition of 0 4 μCi ^{90}Sr in 68 per cent

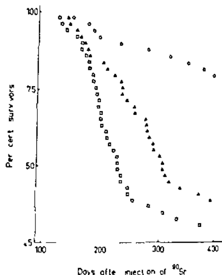


Fig 8 Survival time for mice Treatment with ^{90}Sr, 0 2 $\mu Ci/g$ body weight (O——O) External irradiation 4×35 R (△——△) and 4×70 R (□——□) plus ^{90}Sr, 0 2 $\mu Ci/g$ body weight

lymphoma of the thymus The incidence of non-thymic lymphoma after external irradiation (17 per cent) decreased after additional treatment with ^{90}Sr (to 3 per cent after an addition of 0 4 μCi) After thymectomy the incidence of non-thymic lymphoma increased from 13 to 28 per cent

If the roentgen dose was lowered (from 4 × 140 R to 4 × 70 R) and the dose of strontium remained at 0 2 $\mu Ci/g$ body weight, the incidence of thymic lymphoma decreased from 74 to 46 per cent while the incidence of non-thymic lymphoma slightly increased from 13 to 16 per cent If, however, the dose of external irradiation was lowered further (4 × 35 R), the incidence of non-thymic lymphoma was doubled (from 16 to 32 per cent) at the same time as the thymic lymphoma incidence decreased from 46 to 18 per cent (Table 3) Lymphomas around the vertebral column appeared in six mice (12 per cent) which had received 4 × 70 R + ^{90}Sr and in ten mice (20 per cent) after treatment with 4 × 35 R + ^{90}Sr Among these mice 3 and 4, respectively, had at the same time a thymic lymphoma

Latency time No significant difference in mean latency time appeared between thymic lymphoma and non-thymic lymphoma (Table 3) However, there was a tendency for the latency time of both these tumours to decrease after additional treatment of irradiated (4 × 140 R) mice with ^{90}Sr

Survival time The survival time for mice in different groups is shown in Figs 7 and 8 Combined treatment with roentgen irradiation and ^{90}Sr led to a reduced survival time in comparison to treatment with only external irradiation or only ^{90}Sr

Discussion

Thymus The diphasic regeneration process in the thymus after external whole-body irradiation has been described earlier by JARPLID (1968) A similar regeneration process in the thymus has been found after injection of ^{90}Sr in different doses (JARPLID 1973) After a combination of these forms of irradiation no second regeneration phase occurred in this experiment (Fig 1) Instead the second depletion phase was accentuated and prolonged, so that the weight of the thymus did not attain normal value during the entire observation period Histologically the thymus was characterized during this second depletion phase by a high incidence of bilateral lymphocyte depletion and low incidence of normal appearance Earlier experiments have shown that protection of active bone marrow under external irradiation or injection of viable bone marrow cells after such irradiation promotes the regeneration of the thymus and prevents the occurrence of the second depletion phase (KAPLAN et coll 1953, JARPLID 1968) The role of the protected bone marrow in this respect is not entirely established, but the existing data may indicate that it furnishes stem cells which can repopulate the thymus and facilitate its regeneration (POPP 1961, FORD & MICKLEM 1963, METCALF 1966, WALLIS et coll 1966) This suggests that the absence of recovery of the thymus in this experiment may be associated with a ^{90}Sr induced

asymmetry during the different observation periods was 38, 33 and 54 per cent respectively Inspection and biopsy of the thymus indicate that unilateral thymic changes can alternate from one lobe to the other within the same thymus (reversed asymmetry, JARPLID 1968) The asymmetry thus appears to be an expression of instability within the thymus In this experiment the incidence of asymmetry was highest during the period immediately before the manifestation of lymphoma, which may also indicate that asymmetry and thymic lymphoma are in some way associated phenomena (JARPLID 1968)

After external irradiation and strontium treatment the incidence of asymmetry from day 20 to 100 was around 30 per cent (Fig 2) From day 30 to 54 there was an increase in the incidence of bilateral regeneration, and during the last part of the observation period, from day 55 to 100, lymphoma also appeared, so that the incidences of bilateral regeneration, *asymmetry* and lymphoma were then of similar magnitude (26 to 30 per cent) These various changes were seen in 86 per cent of the animals in this last period and the final incidence of thymic lymphoma was 74 per cent (mean latency time 164 days, Table 3) It thus appears probable that, after this combined treatment, bilateral regeneration in small thymic lobes and asymmetry may be of the same significance for the oc-

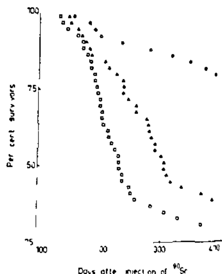


Fig. 8. Survival time for mice. Treatment with ^{90}Sr, 0.2 μCi/g body weight (O——O). External irradiation 4×35 R (△——△) and 4×70 R (□——□) plus ^{90}Sr, 0.2 μCi/g body weight.

lymphoma of the thymus. The incidence of non-thymic lymphoma after external irradiation (17 per cent) decreased after additional treatment with ^{90}Sr (to 3 per cent after an addition of 0.4 μCi). After thymectomy the incidence of non-thymic lymphoma increased from 13 to 28 per cent.

If the roentgen dose was lowered (from 4 × 140 R to 4 × 70 R) and the dose of strontium remained at 0.2 μCi/g body weight, the incidence of thymic lymphoma decreased from 74 to 46 per cent while the incidence of non-thymic lymphoma slightly increased from 13 to 16 per cent. If, however, the dose of external irradiation was lowered further (4 × 35 R), the incidence of non-thymic lymphoma was doubled (from 16 to 32 per cent) at the same time as the thymic lymphoma incidence decreased from 46 to 18 per cent (Table 3). Lymphomas around the vertebral column appeared in six mice (12 per cent) which had received 4 × 70 R + ^{90}Sr and in ten mice (20 per cent) after treatment with 4 × 35 R + ^{90}Sr. Among these mice 3 and 4, respectively, had at the same time a thymic lymphoma.

Latency time. No significant difference in mean latency time appeared between thymic lymphoma and non-thymic lymphoma (Table 3). However, there was a tendency for the latency time of both these tumours to decrease after additional treatment of irradiated (4 × 140 R) mice with ^{90}Sr.

Survival time. The survival time for mice in different groups is shown in Figs 7 and 8. Combined treatment with roentgen irradiation and ^{90}Sr led to a reduced survival time in comparison to treatment with only external irradiation or only ^{90}Sr.

leukemogenically irradiated mice (ILBERRY et coll. 1963, JONEJA & STICH 1965).

After treatment with 4 × 140 R externally or 0.2 μCi ^{90}Sr internally the incidence of non-thymic lymphoma was 17 and 12 per cent respectively (Table 3). The incidence induced by 4 × 140 R (17 per cent) was decreased by injection of strontium. Thus, with an addition of 0.4 μCi strontium, the incidence fell to 3 per cent, probably because the radiation injury to the bone marrow was then too great. NILSSON (1971), in fact, found that the majority of non-thymic lymphomas induced by strontium start in the bone marrow and that, in the dose range 0.2 to 0.8 μCi, the incidence of such bone marrow lymphomas is inversely related to the injected dose. The incidence of non-thymic lymphomas found after treatment with 0.2 μCi ^{90}Sr (12 per cent) could be raised (to 32 per cent) only if the treatment was combined with the small roentgen dose here tested (4 × 35 R). The degree of damage and regeneration so obtained in the bone marrow manifestly favoured the occurrence of lymphoma.

The combination of strontium and a relatively low dose of roentgen induced lymphoma around the vertebrae of some mice. It appears probable that these lymphomas derived from the vertebral marrow, which was assumed by WATANABE (1958) to be the site of predilection for the development of strontium-induced leukaemias. The simultaneous occurrence of thymic lymphoma and of lymphoma deriving from the bone marrow of the vertebral column may indicate primary lymphomas both in thymus and bone marrow. This would in such case explain the doubling of the incidence of non-thymic lymphoma found after thymectomy of animals receiving the combined treatment (from 13 to 28 per cent). ITO et coll. (1969) also found that thymectomy appeared to favour the occurrence of strontium-induced non-thymic leukaemia.

There was no significant difference in mean latency time between thymic lymphoma and non-thymic lymphoma, which is in agreement with an earlier investigation on strontium-induced lymphomas (NILSSON 1971).

Acknowledgement

The excellent technical assistance of B. M. Svedenstål is gratefully acknowledged. The investigation was carried out as part of the programme of the European Late Effects Project Group (EULEP).

SUMMARY

The effect of fractionated external whole body irradiation has been compared with the combined effect of such irradiation and of radiation from ^{90}Sr in respect of changes in the haematopoietic tissue and the development of lymphoma in mice. The investigation has

currence of these thymic lymphomas as are unilateral thymic changes (asymmetry) for the occurrence of thymic lymphoma after external irradiation alone (JARPLID 1968).

Bone marrow The bone marrow changes after fractionated external irradiation conformed with those described earlier (FLIEDNER et coll 1961, JARPLID 1968) The delayed regeneration seen after combined treatment is presumably caused by radiation from strontium accumulated in the bone The fact that during the later part of the observation period the sternum and femur differed in respect of cellularity is probably attributable to the variation within different bones and sections of bone of the dosage of radiation absorbed in the bone marrow owing to differences in metabolism, geometry etc. (NILSSON 1962)

Spleen The increased extramedullary haematopoiesis in the red pulp of the spleen persisted for about three weeks after external irradiation and during the entire observation period after combined treatment This increased haematopoiesis, which has also been seen for long periods after treatment with only ^{90}Sr (NILSSON 1962), is compensatory for the bone marrow injury

Peripheral blood The low number of leucocytes in the peripheral blood during the entire observation period after combined treatment also probably reflects the bone marrow injury by ^{90}Sr (NILSSON 1962)

Lymphoma development The incidence of thymic lymphoma fell with the roentgen dose if the strontium dose was maintained constant (0 2 μCi/g body weight, Table 3) If the animals were not roentgen-irradiated but merely injected with 0 2 μCi strontium, the incidence of thymic lymphoma was 2 per cent, which cannot be said to deviate from the observed spontaneous incidence in this mouse strain (NILSSON 1971) The injury caused to the thymus by this dose of ^{90}Sr appears also to be relatively insignificant (JARPLID 1973)

As previously mentioned, lymphomas induced by ^{90}Sr derive chiefly from the bone marrow, but those induced by fractionated external radiation usually start in the thymus Treatment of externally irradiated animals with ^{90}Sr, however in some cases caused a twofold increase in the incidence of thymic lymphoma It has been mentioned above that the absence of thymic regeneration may be due to a lack of competent bone marrow cells It is possible, too, that the radiation injured thymus is fed with bone marrow cells which, through radiation from strontium, have become defective or modified and which may conceivably be potentially malignant For chromosomally abnormal haematopoietic cells have proved capable of survival in the thymus but not in bone marrow or spleen of

KAPLAN H S Observations on radiation induced lymphoid tumors of mice Cancer Res 7 (1947), 141

— BROWN M B and PAULL J Influence of bone marrow injections on involution and neoplasia of mouse thymus after systemic irradiation J nat Cancer Inst 14 (1953), 303

METCALF D The thymus Recent results in cancer research Springer Verlag, Berlin 1966

NILSSON A Effects of radiostrontium on the blood and haematopoietic tissues of mice Acta vet scand 3 (1962) 103

— Pathologic effects of different doses of radiostrontium in mice Changes in the haematopoietic system Acta radiol Ther Phys Biol 9 (1970), 528

— Pathologic effects of different doses of radiostrontium in mice Development and incidence of leukemia Acta radiol Ther Phys Biol 10 (1971), 115

POPP R A Repopulation of thymus by immunologically competent cells derived from donor marrow Proc Soc exp Biol 108 (1961) 561

ROMEIS B Mikroskopische Technik Leibniz Verlag Munchen 1948

SJODIN K DALMASSO A P, SMITH J M and MARTINEZ C Thymectomy in newborn and

WATANABE S Leucémies expérimentales provoquees chez la souris par administration répétée de petites doses de certains radioisotopes (^{32}P, ^{89}Sr et ^{144}Ce) Pathol Biol (Extrait de Sem Hop Paris) 34 (1958) 1833

shown that the *regeneration seen in* haematopoietic tissues after roentgen irradiation is delayed if the external irradiation is supplemented by ^{90}Sr treatment The delayed bone marrow regeneration leads, inter alia, to incomplete *regeneration and increased incidence* of lymphomas in the thymus Strontium-induced non-thymic lymphomas increase in incidence if ^{90}Sr treatment is supplemented by small doses of external irradiation

ZUSAMMENFASSUNG

Der Effekt fraktionierter externer Ganzkorperbestrahlung wurde mit dem kombinierten Effekt solcher Bestrahlung und der Bestrahlung durch ^{90}Sr hinsichtlich der Änderungen des haematopoietischen Gewebes und der Entwicklung von Lymphomen bei der Maus untersucht Die Untersuchung zeigt, dass die Regeneration in den hamatopoetischen Geweben *nach Rontgenbestrahlung* verzogert ist, wenn die Externbestrahlung *durch* ^{90}Sr Behandlung erganzt wird Die verzogerte Knochenmarksregeneration fuhrt unter anderem zu einer unvollstandigen Regeneration und zu einem erhohten Vorkommen von Lymphomen im Thymus Strontium induzierte nicht-Thymus Lymphome treten gehauft auf, wenn die ^{90}Sr Behandlung durch kleine Dosen externer Bestrahlung erganzt wird

RÉSUMÉ

L'auteur a comparé l'effet de l'irradiation corporelle totale externe fractionnée avec les effets associés de cette irradiation et du rayonnement du ^{90}Sr en ce qui concerne les modifications du tissu hématopoïétique et le développement des lymphomes chez des souris Ce travail a montré que la régénération constatée dans les tissus hématopoiétiques après irradia*tion roentgen est retardée si l'irradiation externe est complétée par un* traitement au ^{90}Sr Le retard de la régénération de la moelle osseuse conduit, entre autres choses, à une régénération incomplète et à une augmentation de la fréquence des lymphomes dans le thymus La fréquence des lymphomes extrathymiques induits par le strontium augemente si le traitement par le ^{90}Sr est complété par de petites doses d'irradiation externe

REFERENCES

FLIEDNER T M, BOND V P and CRONKITE E P: Structural, cytologic and autoradiographic (H^3-thymidine) changes in the bone marrow following total body irradiation Amer J Path *38 (1961), 599*

FORD C E and MICKLEM H S The thymus and lymph nodes in radiation chimaeras Lancet 1 (1963), 359

ILBERRY P L T, MOORE P A, WINN S M and FORD C E Chromosomes in murine preleukemia *In* Cellular basis and aetiology of late somatic effects of ionizing radiation, p *83* Edited by R J C Harris Academic Press, London *1963*

ITO T, YOKORO K, ITO A and NISHIHARA E A comparative study of the leukemogenic effects of strontium-90 and X-rays in mice Proc Soc exp Biol 130 (1969), 345

JARPLID B Radiation induced asymmetry and lymphoma of thymus in mice Acta radiol (1968) Suppl No 279

— Radiostrontium induced early changes in the haematopoietic tissues *With special reference* to the thymus in mice Acta radiol Ther Phys Biol 12 (1973), 145

JONEJA M G *and* STICH H F Chromosomes of tumor cells IV Cell population changes in thymus, spleen, and bone marrow during X-ray-induced leukemogenesis in C_{57} Bl/6J mice J nat Cancer Inst 35 (1965), 421

KAPLAN H S Observations on radiation induced lymphoid tumors of mice Cancer Res 7 (1947), 141
— BROWN M B and PAULL J Influence of bone marrow injections on involution and neoplasia of mouse thymus after systemic irradiation J nat Cancer Inst 14 (1953), 303
METCALF D The thymus Recent results in cancer research Springer-Verlag Berlin 1966
NILSSON A Effects of radiostrontium on the blood and haematopoietic tissues of mice Acta vet scand 3 (1962) 103
— Pathologic effects of different doses of radiostrontium in mice Changes in the haematopoietic system Acta radiol Ther Phys Biol 9 (1970), 528
— Pathologic effects of different doses of radiostrontium in mice Development and incidence of leukemia Acta radiol Ther Phys Biol 10 (1971), 115
POPP R A Repopulation of thymus by immunologically competent cells derived from donor marrow Proc Soc exp Biol 108 (1961) 561
ROMEIS B Mikroskopische Technik Leibniz Verlag Munchen 1948
SJODIN K DALMASSO A P, SMITH J M and MARTINEZ C Thymectomy in newborn and adult mice Transplantation 1 (1963), 521
WALLIS V DAVIES A J and KOLLER P C Inhibition of radiation induced leukemia by the injection of haematopoietic tissue A study of chimaerism Nature 210 (1966), 500
WATANABE S Leucémies expérimentales provoquées chez la souris par administration répétée de petites doses de certains radioisotopes (^{32}P, ^{89}Sr et ^{144}Ce) Pathol Biol (Extrait de Sem Hop Paris) 34 (1958), 1833

FROM THE DEPARTMENTS OF ZOOLOGY AND RADIATION THERAPY, UNIVERSITY OF LUND, S 221 85 LUND, SWEDEN

EFFECTS OF IRRADIATION ON THE CILIA OF THE SYLVIAN AQUEDUCT

A scanning electron microscopic investigation

C VON MECKLENBURG, C H HÅKANSSON and M LINDGREN

The existence of a ciliated surface of the ependymal coating of the ventricular system of the central nervous system has been known for more than a hundred years although few investigations of the physiologic function of the cilia have been reported Beating cilia in the brains of mammals were first revealed by PURKINJE in 1836 VALENTIN (1842) was the first to describe ciliary movements in the ventricular system of the human foetus and adult man LUSCHKA (1855) confirmed these observations while they were contradicted by HASSALL (1852) The ependymal coating of the ventricles of the brain was then largely forgotten until the scanning microscope has again stimulated its examination Most investigations on the ependymal cilia have been later than 1970 (KNIGGE & SCOTT 1970, TORACK & FINKE 1971, CLEMENTE & MARINI 1972, SCOTT et coll 1972) The physiology of these cilia has also been the subject of much discussion SCOTT et coll stated Cilia have traditionally been regarded as structures responsible for the movement of matter (e g mucus, fluids) through the lumen of the tubular organs' That the cilia possess this power was demonstrated by KONNO & SHIOTANI (1956) and CATHCART & WORTHINGTON (1964) who used red blood corpuscles on the ventricular surface to demonstrate the stream caused by ciliary action When the ciliary beating was stopped by epinephrine, the red blood

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corpuscles failed to move A surface coated by cilia, due to its larger area, has a greater power of absorption Microvilli, however, have also been described as being responsible for the function This physiologic aspect has been established for surfaces covered by cilia in other parts of the body, for example in the trachea, nasal sinuses, nasal cavity and the ovarian duct

This communication deals with the response of the cilia to ionizing irradiation in human subjects It describes the appearances of the surface of the aqueduct of Sylvius in a patient who had undergone irradiation treatment for a temporal tumour and who died one year later It is hoped that it will be of value to those who treat cerebral tumours and who have access to both scanning electron microscopy (SEM) and transmission electron microscopy to perform investigations of this type It is obvious that the relatively restricted employment of this method necessitates collecting data from the isolated cases in different parts of the world in order to reach any conclusive results It is important to establish a standardized method of approach as a basis for later comparisons

There were two reasons for choosing the aqueduct of Sylvius for examination First, it has been demonstrated that the aqueduct contains ciliated cells and since the tubulus produces a relatively high speed of ventricular fluid transportation, it was considered that the cilia had to be physiologically active Secondly, the area received a large dose of irradiation so that abnormalities could be considered as being due to its effects

Materials and Methods A man, aged 75, who had died at the same time as the irradiated patient, was selected as a control The sample was taken 21 hours after death in both subjects The control had died from bronchopneumonia The irradiated patient was a woman aged 62 who had had headache for a year EEG, pneumography and angiography revealed a tumour of the right temporal lobe Operation disclosed that the intracranial pressure was considerably raised with the gyri sulci levelled and destroyed The corresponding upper posterior part of the temporal lobe fluctuated on palpation One centimetre from the surface a cyst was emptied of a yellowish fluid A reddish blue solid tumour at the bottom of the cyst with the appearances of a malignant glioma infiltrated medially and upwards into the posterior part of the frontal lobe Some of the mass was sucked out, microscopy revealed malignant glioma

The condition of the patient markedly improved It was the intention to give 5 600 to 5 800 rad within the area of the tumour and about 4 500 rad in the midline Calculations indicated that this could be performed from only one side As the tumor was so large, it was necessary that the field towards the site of the tumour should have a good margin The patient was admitted to the radiation therapy clinic A field 9 cm × 10 cm in size over the temporal lobe was mapped

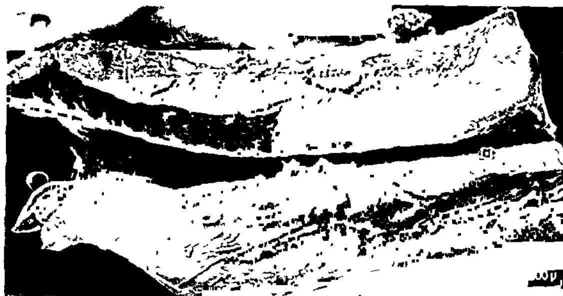


Fig. 1. Scanning electron microscopy of sectioned aqueduct of Sylvius. Cranial part to the left. ×17.5

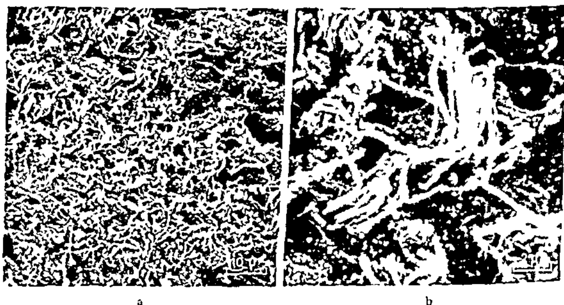


Fig. 2. Scanning electron microscopy from area A in Fig. 1 with groups of cilia. a) ×890 b) ×4 440

out from the angiography with the aqueduct of Sylvius in the midline and near the midpoint of the field, and Betatron irradiation with a 33 MV dose of 1 625 rad (19 days, 10 fractions, 5 days a week) applied. As the field was large, only 75 rad were given at the beginning and successively raised to 225 rad. This treat-



Fig 3 Scanning electron microscopy from area B in Fig 1 with groups of cilia × 5 520

ment was immediately followed by 8 MV photons over 23 days (total of 11 fractions with 225 rad at each treatment except the last two that were each of 250 rad) This scheme was interspersed with the administration of electrons (over 41 days), from 200 rad at the beginning up to 275 rad The patient received a total calculated maximum dose of 5 800 rad to the tumour over 48 days and died 12 months later

Both the cranial end and the caudal part of the aqueduct lay within the field of the full dose' which was calculated to be 4 800 rad in the midline (at a depth of 7 cm)

The aqueduct of Sylvius of the control was cut out in a rectangular cube measuring 7 mm × 7 mm × 17 mm, and after pre preparation a part of it was fixed in glutaraldehyde 2 5 % in phosphate buffer (MILLONIG 1961), Ph = 7 4 for two hours After rinsing in pure phosphate buffer, the specimen was divided in its midline and dehydrated in acetone, amylacetate and liquid carbon dioxide, and finally dried by the critical point method of ANDERSSON (1951) The pieces were then attached to a frame and coated with gold palladium, after which they were examined microscopically with a Cambridge—Mark II Stereoscan scanning electron microscope



Fig 1 Scanning electron microscopy of sectioned aqueduct of Sylvius Cranial part to the left ×17 5

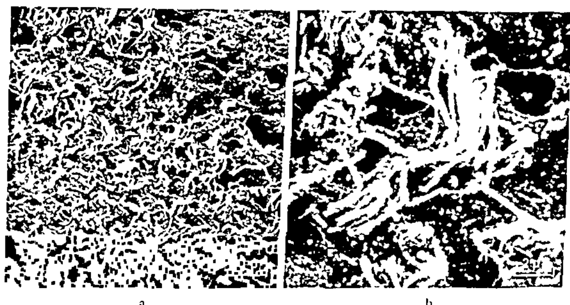
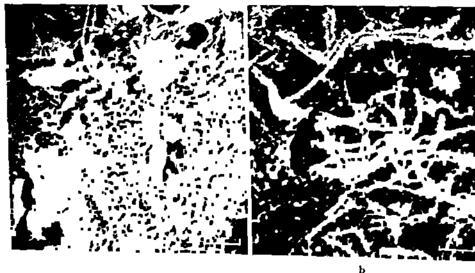


Fig 2 Scanning electron microscopy from area A in Fig 1 with groups of cilia a) ×890 b) ×4 440

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Fig 5 Scanning electron microscopy of sectioned aqueduct of Sylvius from irradiated patient Cranial part to the left ×19 5



b

Fig 6 Scanning electron microscopy from area A in Fig 5 with groups of cilia a) ×935 b) ×4 680

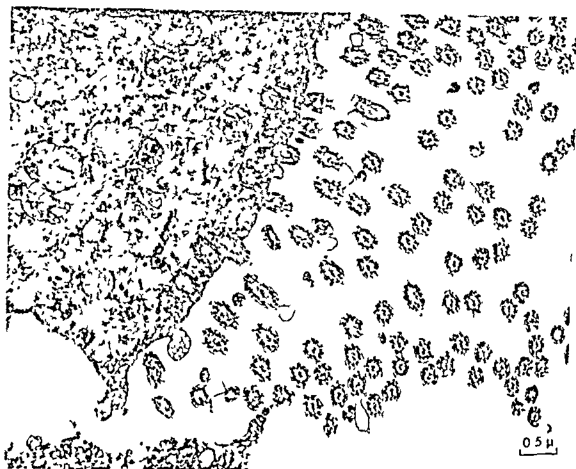


Fig. 4. Transmission electron microscopy of the ependymal ciliated cells in length and cross sectioned 9 + 2 cilia. × 17 500.

Parts of the epithelium of the aqueduct of Sylvius were then fixed in OsO_4 1 % in phosphate buffer, Ph = 7.4 for two hours, after which they were rinsed in pure buffer, dehydrated in alcohol and imbedded in Vestopal W with styrene. One micron thick section was dyed in Richardson's azure II. Ultra-thin slices were cut out on an LKB-Ultrotome and contrasted in lead acetate-uranyl-acetate or in a cube with uranylacetate 0.5 %. They were then examined by microscopy with a Philips EM 300 electronmicroscope.

Results

One half of the sectioned aqueduct of Sylvius appears in Fig. 1. The ependymal cells covering the aqueduct were ciliated (Figs 2 a, 3). The cilia lay in groups and appeared to be evenly distributed along the entire aqueduct. The small, round formations resembling microvilli in Fig. 2 b probably represent postmortem

No further conclusions may be drawn about the effects of irradiation on the cilia in the current case. The cilia in the whole of the aqueduct of Sylvius had received 4 800 rad, but did not appear to differ much from those from the control patient. Experience with ciliary surfaces of the type in, for example the trachea and the bronchi, have indicated that cilia have an enormous regenerative capacity, so that those in the present examinations may have been newly developed cilia, unaffected by irradiation given 12 months earlier. A much larger material is necessary before any statements can be made regarding the effects of irradiation on the dynamics of the cerebrospinal fluid space.

SUMMARY

Scanning electron microscopy has been used to examine the surface of the aqueduct of Sylvius in a patient irradiated following operation for a malignant temporal glioma. The number of cilia covering the surface were reduced in comparison with those of a non irradiated patient. The effect upon the circulation of the ventricular fluid is discussed.

ZUSAMMENFASSUNG

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RÉSUMÉ

La microscopie electronique à balayage a ete utilisee pour examiner la surface de l'aqueduc de Sylvius *chez un malade irradie* apres operation pour un gliome temporal malin. Le nombre de cils couvrant la *surface* etait diminue par rapport à un sujet non irradie. Les auteurs examinent l'effet de cette lèsion sur la circulation *du liquide* ventriculaire.

REFERENCES

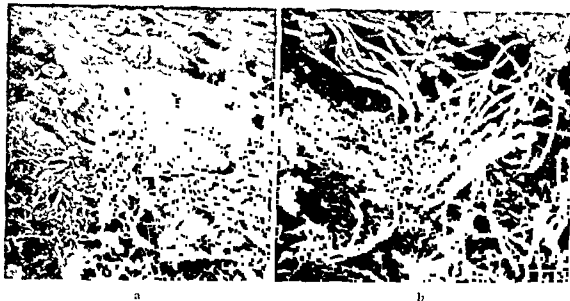
ANDERSSON, T. F. Techniques for the preservation of three *dimensional* structure in prepa[illegible] specimens for the ele[illegible]

CATH[illegible] ele[illegible]

CLEM[illegible] [illegible] of the walls of cerebral ventricles and of choroid plexus in cat. *Z. Zellforsch.* 123 (1972), 82.

HASSALL, A. H. Mikroskopische Anatomie des *menschlichen* Körpers im gesunden und kranken Zustande. 1852.

KNIGGE, K. M. and SCOTT, D. E. Structure and function of the median eminence. Amer. J. Anat. 129 (1970), 223.



a b

Fig 7 Scanning electron microscopy from area B in Fig 5 with groups of cilia a) ×835 b) ×4 170

changes. Fig 4 is a section through the ependymal cells; they belong to the 9 + 2 type of cilia, the motile ones Fig 5 represents one half of the sectioned aqueduct of Sylvius taken from the irradiated patient, where the ependymal cells are also ciliated, but where areas with a lesser number of cilia and also parts having no cilia at all are present (Figs 6 a, 7 a). It is not possible to ascertain any differences in the appearance and thickness of the cilia The ependymal cells (Fig 7 b) also have surface formations resembling microvilli, and are again probably postmortem changes.

Discussion

The presentation although only of a single case would appear to be justified by introducing radiation biology, and more specifically the therapy of tumours of the central nervous system, into more or less virgin domains. Virgin, as the instruments necessary for the examinations have not previously been available although not unknown, for knowledge about the cilia has existed for a very long time

The scanning electron microscope findings might indicate a generally reduced number of ciliary cells and it is assumed that this might have been the consequence of the radiation therapy, the circulation of the ventricular fluid might thus have been affected

No further conclusions may be drawn about the effects of irradiation on the cilia in the current case. The cilia in the whole of the aqueduct of Sylvius had received 4 800 rad, but did not appear to differ much from those from the control patient. Experience with ciliary surfaces of the type in, for example the trachea and the bronchi, have indicated that cilia have an enormous regenerative capacity, so that those in the present examinations may have been newly developed cilia, unaffected by irradiation given 12 months earlier. A much larger material is necessary before any statements can be made regarding the effects of irradiation on the dynamics of the cerebrospinal fluid space.

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ZUSAMMENFASSUNG

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RÉSUMÉ

La microscopie *électronique à balayage* a été utilisée pour examiner la surface de l'aqueduc de Sylvius chez un *malade irradié* après opération pour un gliome temporal malin. Le nombre de cils couvrant la *surface* était diminué par rapport à un sujet non irradié. Les auteurs examinent l'*effet de cette lésion sur* la circulation du liquide ventriculaire.

REFERENCES

ANDERSSON T F *Techniques for the* preservation of three dimensional structure in preparing specimens for the elect[illegible]

CATHCART F [illegible] clearing [illegible]

CLEMENTE F [illegible]

HA[illegible]

KNIGGE K M and SCOTT D E *Structure* and function of the median eminence. Amer J Anat 129 (1970) 223

KONNO I and SHIOTANI Y Some aspects on the ciliary movement of ependymal cells of the ventricles Folia psychiat neurol jap 10 (1956), 1

LUSCHKA H Adergeflechte des menschlichen Gehirns Druck und Verlag von Georg Reimer Berlin, 1855

PURKINJE J E Über Flimmerbewegungen im Gehirn Mullers Archiv Anat Physiol 1836

SCOTT D E, KOZLOWSKI G P and KROBISCH DUDLEY G A comparative ultrastructural analysis of the third cerebral ventricle of the North American Mink (Mustala vison) Anat Rec 175 (1973), 155

— PAULL W K and KROBISCH DUDLEY G A comparative scanning electron microscopic analysis of the human cerebral ventricular system Z Zellforsch 132 (1972) 203

TORACK R M and FINKE L H Evidence for the sequestration of function within area postrema based on scanning electron microscopy and the penetration of horse radish peroxidase Z Zellforsch 118 (1971), 85

VALENTIN G Flimmerbewegung Wagners Handworterbuch d Physiol, Bd 1, Braunschweig 1842

WORTHINGTON W C and CATHCART R S Ependymal cilia Distribution and activity in the adult human brain Science 139 (1963), 221

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THERMAL SENSITIVITY AND THE EFFECT OF ELEVATED TEMPERATURES ON THE RADIATION SENSITIVITY OF CHINESE HAMSTER CELLS

J E ROBINSON and M J WIZENBERG

The use of both elevated and reduced temperatures as radiation modifiers in clinical radiation therapy has a long history and there is appre[illegible] the literatu[illegible] protection [illegible] and that elevated temperatures may produce increased sensitivity to ionizing radiation This evidence includes laboratory experiments with model tumor systems (CRILE 1967) and cells cultured in vitro (CHAMBERLAIN 1967) Although the bibliographies on this subject are massive, much of the work is of such a nature that it is difficult to draw firm conclusions and generate any clear picture regarding the relationship between elevated temperatures and the radiation sensitivity of mammalian cells This is in part attributable to the fact that much of the work was done without adequate quantitation *However, the mass of* suggestive data is sufficient, we feel, to justify a thorough investigation of hyperthermia plus ionizing radiation as a possible combined mode of cancer therapy This report is one part of such an investigation intended to elucidate (1) the

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thermal sensitivity of a strain of Chinese hamster cells and (2) the effect of hyperthermia on the radiation sensitivity of these cells

Material and Methods

Cell techniques The cells are a substrain of Chinese hamster lung cells originally obtained from the laboratory of Dr Elkind of the National Institutes of Health Stock cultures are routinely grown in glass prescription bottles with Eagle's medium supplemented with 15 to 20 per cent of fetal calf serum and 10 per cent of NCTC 109 medium

Stock cultures are subcultured with the aid of trypsin, single cell suspensions are prepared, suitably diluted with medium, and placed in siliconized screw-cap test tubes for either thermal or radiation treatments

Thermal treatment For the thermal inactivation experiments, test tubes of cell suspensions were placed in precision controlled water baths for periods of time ranging up to five hours To avoid cell adherence and clumping, the test tubes were periodically agitated with a commercial 'vortex' stirrer In a given experiment, all test tubes (representing different treatment times) were simultaneously placed in the elevated temperature bath, then the tubes receiving shorter treatment times were sequentially removed and placed in a control water bath at 37 5° C where they were accumulated until the longest treatment time had elapsed These cell suspensions were then used to plant clonal assay cultures in plastic petri dishes, which were incubated at 37 5° C until colonies reached sufficient size for counting

Combined thermal and radiation treatment The approach was to adopt a particular elevated temperature regimen, determine a radiation survival curve for cells subjected to the combined treatment, and then compare the survival curves obtained using elevated temperatures to those obtained from cells subject to the same treatment, but at 37 5° C The thermal treatment was designed to be one which produced marked thermal damage, but not massive thermal cell killing

Since there was no prior knowledge regarding the importance of irradiation and thermal treatment sequence, the experiments were designed to maximize the likelihood of detecting thermal radiation sensitization regardless of sequence The treatment regimen was as follows The cells were maintained in suspension in test tubes at temperatures of 37 5, 40, 41 and 42° C for a total period of two hours The thermal treatment was initiated before irradiation, maintained during irradiation, and continued after irradiation (2 h PDP) The thermal treatment time was equally divided between the pre-irradiation and post irradiation treat-

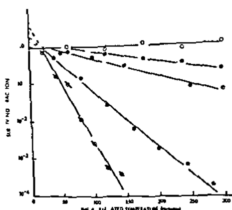


Fig 1

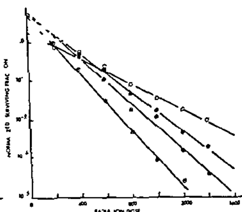


Fig 2

Fig 1 Thermal survival curves for Chinese hamster cells ○ 40 0 ● 42 0 ○ 42 5 ○ 43 0 ⌀ 44 0 C

Fig 2 Radiation survival curves for Chinese hamster cells subjected to thermal treatment (2 hours before during and after irradiation) The curves are normalized to a surviving fraction of 1 0 at zero radiation dose (rad) ○ 37 5 ● 40 0 ⊖ 41 0 ○ 42 0° C

ment periods Upon the completion of the post irradiation thermal treatment, the cells were planted in petri dishes and incubated at 37 5° C until the colonies grew large enough to be scored

The cells were irradiated with 250 kVp conventional radiation therapy unit in a temperature controlled lucite water bath irradiation chamber The dose rate was approximately 100 rad per minute Dosimetry was determined by means of an NBS calibrated Victoreen dosimeter

Results

Thermal sensitivity The thermal inactivation curves obtained are shown in Fig 1 The surviving fraction on a log scale is plotted against time at the elevated temperatures for treatment temperatures of 40, 42, 42 5, 43 and 44° C The curves are exponential at large treatment times but with a shoulder—almost identical in form to classic radiation survival curves There is net growth at 40° C some killing at 42°, with a rapid increase in the rate of thermal killing with increasing temperatures The lines are best fit lines to the survival data beyond the shoulder and are summarized in numerical form in Table 1 Column 3 of that table lists values determined for T_0—a direct analogy to the D_0, the mean lethal dose of radiation survival curves At 42 degrees, 163 minutes are

Table 1

The thermal sensitivity of Chinese hamster lung cells. T_0 is the time necessary to reduce the surviving fraction by a factor of 1/e

Temp °C	Slope ×10²/min	T_0 (1/t) minutes
40.0	+0.07 ± 0.03	Net Growth
42.0	0.61 ± 0.06	163
42.5	1.02 ± 0.06	98
43.0	3.28 ± 0.06	30.4
44.0	6.88 ± 0.25	14.5

required to reduce the surviving fraction of cells by a factor of 1/e, this time decreases to approximately 14 minutes as the temperatures is increased to 44° C.

Effects of hyperthermia on radiation sensitivity. Radiation survival curves which were determined when the cells were subjected to the combined regimen (2 h PDP) at treatment temperatures of 37.5, 41.0, 42.0 and 42.5° C are shown in Fig. 2. Here is plotted the ratio of cells surviving a given radiation dose to those subjected to the same thermal treatment but sham irradiated, versus the radiation dose. The ordinate is labelled "Normalized surviving fraction" to emphasize that the curves have been normalized to yield a surviving fraction of 1.0 at zero radiation dose for all treatment groups and do not reflect thermal killing per se (see Fig. 1). The solid lines are best fit lines to the data which, together with a statistical summary, are given in Table 2. In this table the slopes and their associated standard deviations are enclosed within double lines along the diagonal. The remainder of the table answers questions regarding the values calculated for Student's t test and the level of significance for the differences in slopes found for the various temperature treatments. For example, the t value (25) calculated for the difference in the slope for the treatment at 40° and the 37.5° control is found in the table at the intersection of the column labelled 37.5° C and the row labelled 40.0° C. This t value of 25 indicates that the difference is significant at the 99.95 per cent level of confidence.

Effect of heating time, the heating–irradiation sequence, and the delay between heating and irradiation. Experiments were conducted on the effect of heating time at 42° C (with 37.5° C controls) and the effect of sequence in which the temperature treatment and irradiation were applied. The primary goal in designing these experiments was to supply practical information relevant to the logistics of tumor experiments with small animals and, eventually, patient treat-

Table 2

The effect of thermal treatment on radiation sensitivity as indicated by survival curve slope, B. When the higher temperature has a greater radiation sensitivity it is indicated by 'yes'

	42.0° C	41.0° C	40.0° C	37.5° C
42.0° C	B ± σB 0.85 ± 0.01*	YES t = 10** 99.95 %	YES t = 20 99.95 %	YES t = 41 99.95 %
41.0° C		B ± σB 0.68 ± .02	YES t = 6.5 99.95 %	YES t = 14 99.95 %
40.0° C			B ± σB 0.57 ± .01	YES t = 25 99.95 %
37.5° C				B ± σB 0.39 ± 0.005

* Slopes have units of 1/100 rad
** Value for Student's t test

Table 3

Treatment scheme

Treatment			Time (min)				
			0	15	30	60	120
A	Before irradiation	Heating time at 42° C		—	—	—	
B	After irradiation			—	—	—	
C	Before, during and after irradiation						—
D	One hour heating before irradiation	Delay time at 37.5° C	—	—	—	—	
E	Controls		Maintained at 37.5° C				

ment. The combinations of treatment applied in this series of experiments are given in Table 3.

Some results obtained involving treatment groups *A*, *B*, *C* and *E* are shown in graphic form in Fig. 3, in which is plotted the slope of survival curves versus heating time at 42° C. The point at zero heating time represents the 37.5° C

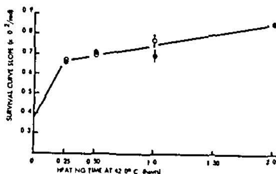


Fig 3

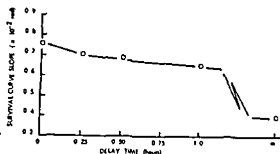


Fig 4

Fig 3 The effect of heating time and the heating irradiation sequence on radiation sensitivity treatment temperature of 42° C ○ After irradiation ● before irradiation ⊕ before after and during irradiation

Fig 4 Effect of delay between heating and irradiation on radiation sensitivity (survival curve slope) The thermal treatment was one hour at 42° C

control The data suggest (1) It is not necessary to maintain the elevated temperatures during irradiation in order to achieve thermal sensitization, (2) thermal sensitization is achieved when the thermal treatment is applied either before or *after irradiation, and the degree of sensitization is approximately the same*, (3) appreciable thermal sensitization is achieved with heating times as short as 15 minutes, but there is some apparent increase in sensitization with longer heating times

Data obtained regarding the effect of delay between heating and irradiation *is shown in Fig 4 In these experiments the cells were heated for one hour at 42* degrees, then placed at 37 5 degrees for various delay periods before irradiation The broken line at the right margin marked represents the normal radiation sensitivity at 37 5° C to which one assumes the radiation sensitivity must return after long delay times The degree of thermal sensitization appears to decrease *with delay interval between the thermal and irradiation treatments but slowly* a major part of the sensitization achieved with the combined treatment remains after a delay period of one hour

Discussion

Thermal sensitivity The similarities in shapes of thermal and radiation survival curves for mammalian cell cultures have also been noted by Harris (1967) and by Westra et coll (1971), although this similarity should not necessarily be constructed to imply similarity in action of the two modes of injury Regarding thermal sensitivity per se The limited data available suggest (at least in vitro) that there may be greater differences in thermal sensitivities for different cells

than for radiation sensitivities At 44° C T_0 values of 54 6 minutes and 4 0 minutes were reported for pig kidney and Chinese hamster ovary cells (HARRIS, WESTRA et coll) compared to an intermediate value of 14 5 for the strain of Chinese hamster lung cells used for these investigations In the context for which these particular investigations were conducted, the thermal treatment time of two hours is 'mild', producing approximately 50 per cent lethality at 44° C—the most drastic thermal treatment, with little or no lethality at 40° C

Effect of hyperthermia on radiation sensitivity The data of Table 2 clearly indicate that there is synergistic action between the effects of elevated temperature and ionizing radiation on cell killing At a temperature of 40° C the radiation sensitivity, as judged by survival curve slope, is greater than that at 37 5 by a factor of 1 5, which increases to 2 2 as the treatment temperature is raised to 42 0° C BELLI et coll (1963), in an investigation primarily directed towards the effects of hypothermia, reported that the D_0 of HeLa cells was decreased from 121 rad at 37 5° C to 95 rad at 40° C The temperature treatment was one in which " cells were exposed for a short time before and during irradia tion " CHAMBERLAIN et coll (1966) report in an abstract that sensitization was achieved at elevated temperatures with both L and HeLa cells and that the L cells were more sensitive to thermal treatment than the HeLa cells In an unpublished Ph D dissertation, CHAMBERLAIN (1967) presented data supporting the concept that the combined effects of thermal and radiation damage are more than additive

The fact that appreciable thermal sensitization is achieved with the temperatures as low as 40 or 41° C lends encouragement to the idea that a treatment regimen combining radiation and hyperthermia might be feasible

Effect of heating time and heating irradiation sequence The data summarized in Fig 3 are again favorable to the practical logistics of a potential combined treatment They suggest that the thermal treatment time need not be long to bring about thermally enhanced sensitivity, and that it may not be necessary to apply the thermal and radiation treatments simultaneously These facts, if generally applicable, mean that one can appreciably enhance radiation sensitivity by short thermal treatments at slightly elevated temperatures The flexibility in the sequence of heating and irradiation would further simplify clinical application

Acknowledgement

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SUMMARY

Thermal survival curves for *Chinese Hamster cells* are exponential with a shoulder. There is net proliferation at 40° C with a rapid increase in thermal sensitivity above that temperature. There is synergistic action between the lethal effects of mild thermal treatments and irradiation. Significant sensitization occurs at 40° C, while cells treated at 42° C are more than twice as radiation sensitive as normal temperature controls. It is not necessary to heat *during irradiation to achieve thermal radiation sensitization*.

ZUSAMMENFASSUNG

Wärme Überlebenskurven von chinesischen Hamsterzellen sind exponentiell mit einer Schulter. Es liegt eine reine Proliferation bei 40° C *mit einem raschen Anstieg in der* Wärmeempfindlichkeit bei Temperaturen darüber vor. Die Letaleffekte einer leichten Wärmebehandlung und einer Bestrahlung sind synergistisch. Eine signifikante Sensibilisierung erfolgt bei 40° C, während bei 42° behandelte Zellen doppelt so strahlenempfindlich sind als Kontrollen bei normaler Temperatur. Es ist notwendig, während der Bestrahlung zu erwärmen, um eine Wärme Strahlensensibilisierung zu erreichen.

RÉSUMÉ

Les courbes de survie à la chaleur des cellules de hamster chinois sont exponentielles avec un épaulement. Il y a une nette prolifération à 40° C avec une rapide augmentation de la sensibilité thermique au dessus de cette température. Les effets léthaux d'exposition modérée à la chaleur et d'irradiation ont une action synergique. Une sensibilisation importante se produit *à 40° C alors que les cellules traitées à 42° C sont plus de deux fois plus sensibles* aux radiations que les cellules témoins à température normale. Il n'est pas nécessaire de les chauffer au cours de l'irradiation pour obtenir une sensibilisation thermique aux radiations.

REFERENCES

BEL [illegible] an

CH [illegible] lls
in culture. A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Medical Physics (Radiology) at the University of California, Los Angeles (1967)

—

CRILE G. The effects of heat and radiation on cancers implanted on the feet of mice. [illegible] Res 23 (1967) 372

HALL D R, LEWIS J D, TOWN C D, FISH P J and LINDROP P J. Radiation response of HeLa cells exposed at very low temperatures. Int J Radiat Biol 16 (1969) 43

HARRIS M. *Temperature resistant variants in clonal populations of pig kidney cells*. Exp Cell Res 46 (1967) 301

WESTRA A and DEWEY W C. Variation in sensitivity of heat shock during cell culture of Chinese hamster cells in vitro. Int J Radiat Biol 19 (1971) 467

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LIVER AND SPLEEN IN RATS AFTER INTRAVENOUS INJECTION OF LIPIODOL ULTRA-FLUIDE AND ^{99}Tc^{m}-SULPHIDE COLLOID

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and Y NAVERSTEN

The occurrence of noncaseating tubercle-like granulomas within otherwise uninvolved organs in Hodgkin's disease has been stated to be relatively frequent (KADIN et coll 1970) These authors considered the granulomatous reaction to represent a distinct phenomenon, possibly related to the altered delayed hypersensitivity known to occur in this condition The lesions could be distinguished from those in lymph nodes after lymphography and occur also in patients not so examined BRINCKER (1970) in investigating 9 patients with Hodgkin's disease and sarcoid like granulomas considered the possibility of a common etiologic factor in the form of altered immune reaction Other types of non specific hepatic reactions in Hodgkin's disease have been described by OEHLERT & DISCHLER (1972) and BAGLEY et coll (1972), these ranged from a simple diffuse activation of the reticulo-endothelial system to a reactive granulomatous hepatitis

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Lymphography and isotope examinations have been widely employed in the staging of patients with Hodgkin's disease A possibility *may exist that the* agents used in these examinations may cause tissue reactions and theoretically could be responsible, at least partly, for the non specific splenic and hepatic *lesions that occur*

Dalton et coll (1965) demonstrated Lipiodol Ultra-Fluide within the reticuloendothelial cells and hepatocytes of the liver after lymphography, Wallace (1967) reported acute *cholangitis with polymorphonuclear infiltrate and occasional* brown globules in the liver of a patient who died subsequent to lymphangiography The possibility that the contrast medium may reach the liver by lymphovenous anastomoses or lympholymphatic anastomoses with tumour involvement of the retroperitoneal lymph nodes has been discussed by Georgi (1967), Gilly & Tafnzer (1967) and Hecht et coll *(1968)*

Numerous particulate materials, injected intravenously, are accumulated in the cells of the reticuloendothelial system Such particles, labelled with different isotopes, for instance ^{198}Au, ^{131}I, ^{99}Tc^{m} and ^{113}In^{m}, have been widely used for diagnostic purposes, especially in scintigraphy of the liver and spleen A search *of the literature has revealed no report of reactions on the cellular level of such* agents

The purpose of the present investigation was to ascertain whether granulomas similar to those reported in Hodgkin's disease would appear in the liver and spleen of rats after injection of Lipiodol Ultra-Fluide alone or combined with ^{99}Tc^{m}-sulphide colloid Granulomas have been observed in the liver and spleen of patients with Hodgkin's disease but with a normal lymphogram and the possibility thus remains that the lymphographic diagnostic material may reach the liver and spleen by the thoracic duct, the lesser circulation and the arterial route The decision was therefore taken to inject the Lipiodol and the ^{99}Tc^{m}-*sulphide colloid intravenously*

Material and Methods After skin incision the right or left jugular veins of white rats weighing 200 g were injected under anaesthesia with a 27 gauge needle The animals were divided into two main groups Group I, consisting of 12 rats, was injected with 0 1 ml Lipiodol Ultra-Fluide The 9 rats of Group II were injected with 0 1 ml Lipiodol Ultra-Fluide and 0 1 ml ^{99}Tc^{m}-sulphide colloid prepared by the method of Persson & Naversten *(1970) A common dose in* clinical practice contains about 3 mg particulate matter Dilution with NaCl 0 9 % permitted the particles in the ^{99}Tc^{m}-sulphide colloid in the dose to the rats to be reduced by a factor of 100 in relation to the diagnostic dose normally used in man the skin incision was then sutured, the animals received normal laboratory food and water

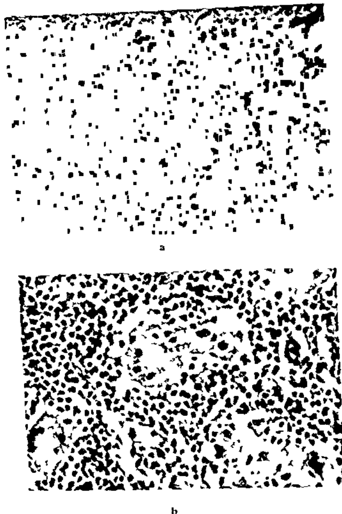


Fig 1 a) Lymphocytes and multinuclear giant cells in the hilar region of the lung in a rat injected with Lipiodol Ultra Fluide Htx eosin × 160 b) Small vacuoles in the cytoplasm of the giant cells Htx eosin × 400

Six weeks after injection 5 animals from group I and 4 from group II were killed, followed two weeks later by the remaining animals Ten normal rats of the same age and weight acted as controls Autopsy The lungs and mediastinum, kidneys, spleen, mesenteric lymph nodes and liver were fixed in formalin 10 % Paraffin blocks were cut at 7 μ and stained with hematoxylin-eosin (van Gieson) Additional staining for acid-fast organisms was sometimes included

Lymphography and isotope examinations have been widely employed in the staging of patients with Hodgkin's disease. A possibility may exist that the agents used in these examinations may cause tissue reactions and theoretically could be responsible, at least partly, for the non-specific splenic and hepatic lesions that occur.

DALTON et coll. (1965) demonstrated Lipiodol Ultra-Fluide within the reticuloendothelial cells and hepatocytes of the liver after lymphography, WALLACE (1967) reported acute cholangitis with polymorphonuclear infiltrate and occasional brown globules in the liver of a patient who died subsequent to lymphangiography. The possibility that the contrast medium may reach the liver by lymphovenous anastomoses or lympholymphatic anastomoses with tumour involvement of the retroperitoneal lymph nodes has been discussed by GROKET (1967), GILLY & TATNAZIR (1967) and HECHT et coll. (1968).

Numerous particulate materials, injected intravenously, are accumulated in the cells of the reticuloendothelial system. Such particles, labelled with different isotopes, for instance ^{198}Au, ^{131}I, ^{99}Tc^{m} and ^{113}In^{m}, have been widely used for diagnostic purposes, especially in scintigraphy of the liver and spleen. A search of the literature has revealed no report of reactions on the cellular level of such agents.

The purpose of the present investigation was to ascertain whether granulomas similar to those reported in Hodgkin's disease would appear in the liver and spleen of rats after injection of Lipiodol Ultra-Fluide alone or combined with ^{99}Tc^{m}-sulphide colloid. Granulomas have been observed in the liver and spleen of patients with Hodgkin's disease but with a normal lymphogram, and the possibility thus remains that the lymphographic diagnostic material may reach the liver and spleen by the thoracic duct, the lesser circulation and the arterial route. The decision was therefore taken to inject the Lipiodol and the ^{99}Tc^{m}-sulphide colloid intravenously.

Material and Methods. After skin incision the right or left jugular veins of white rats weighing 200 g were injected under anaesthesia with a 27 gauge needle. The animals were divided into two main groups. Group I, consisting of 12 rats, was injected with 0.1 ml Lipiodol Ultra-Fluide. The 9 rats of Group II were injected with 0.1 ml Lipiodol Ultra-Fluide and 0.1 ml ^{99}Tc^{m}-sulphide colloid prepared by the method of PERSSON & NAVERSTEN (1970). A common dose in clinical practice contains about 3 mg particulate matter. Dilution with NaCl 0.9 % permitted the particles in the ^{99}Tc^{m}-sulphide colloid in the dose to the rats to be reduced by a factor of 100 in relation to the diagnostic dose normally used in man. The skin incision was then sutured, the animals received normal laboratory food and water.

alveolar phagocytes a possibility existed that it may have been transported to the hilar regions and elicited a foreign body reaction of the type that appeared in 2 of the rats The occurrence of small fat vacuoles in the cytoplasm of the giant cells is in favour of this assumption A cellular reaction with foreign body type giant cells has been described in human lungs after lymphography (HALLGRIMSON & CLOUSE 1965)

DALION et coll (1965) stated that Lipiodol remained for some time in the Kupffer cells and the liver cells During the first 15 days after the injection the fat of the injected Lipiodol was visible as small droplets in the cytoplasm of the affected cells, oil embolism to the liver has been described as a complication of lymphography in man (LEE 1967 and HECHT et coll 1968) It is possible that the cellular infiltrates in the portal triads and the focal cellular aggregates in the liver parenchyma in the present material were due to the Lipiodol accumulated in the liver and that similar lesions in human subjects with Hodgkin's disease have the same origin Their anatomic character, however, is not specific and other causes must also be considered

The frequency of lesions was the same in the two groups and no additional changes were observed when $^{99}Tc^{m}$-sulphide colloid was added to the Lipiodol

SUMMARY

Microscopy of 19 rats after the injection of Lipiodol Ultra Fluide alone or with $^{99}Tc^{m}$ sulphide colloid revealed small aggregates of lymphocytes and sparse portal cellular infiltrates in the liver of 7 of the animals Giant cell granulomas were evident in the hilar regions of the lungs in 2 rats no changes were present in the spleen lymph nodes or kidneys No sarcoid like granulomas were observed

ZUSAMMENFASSUNG

Die mikroskopische Untersuchung von 19 Ratten nach Injektion von Lip dol Ultra Fluide alleine oder mit $^{99}Tc^{m}$-Sulphid Kollo d liess kleine Aggregate von Lymphozyten und Histiozyten und geringe portale zellulare Infiltrate in der Leber von 7 der Tiere erkennen Riesenzellgranulome waren in der Hilusregion der Lungen von 2 Ratten vorhanden es lagen keine Veranderungen in der Milz den Lymphknoten oder den Nieren vor Es wurden keine Sarcoid ahnlichen Granulome beobachtet

RÉSUMÉ

L etude microscopique de 19 rats après injection de Lipiodol Ultra Fluide seul ou avec un collo de de sulfite de $^{99}Tc^{m}$ a montre de petits agregats de lymphocytes et d histiocytes et des inf ltrats cellulaires portaux dissem nes dans le foie de 7 de ces animaux Il y avait des granulomes à cellules geantes évidents dans les regions hilaires des poumons de 2 rats il n y avait pas de mod fications dans la rate, les ganglions lymphatiques ou les reins Il n y avait pas de granulome d aspect sarcoide

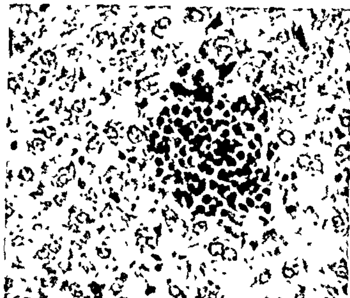


Fig 2 Aggregate of lymphocytes and single histiocytes in the liver of a rat injected with Lipiodol Htx eosin × 400

Results and Discussion

The macroscopic appearances were normal Microscopically one rat in group I and one in group II had single granulomas in the hilar regions of the lungs consisting of aggregates of multinuclear giant cells and lymphocytes (Fig 1) The cytoplasm in some of the giant cells contained small vacuoles similar to those appearing after dissolved fat. Special stains for microorganisms produced negative results No granulomas were evident in the other parts of the lungs

Four of the 12 rats in group I and 3 of the 9 rats in group II had small aggregates of lymphocytes and histiocytes in the hepatic parenchyma (Fig 2) In addition, sparse portal cellular infiltrates were present in 5 of the rats in group I and 3 of the rats in group II, these were composed of lymphocytes and histiocytes No correlation existed between the occurrence of these infiltrates and the circumscribed lymphohistiocytic aggregates No giant cells similar to those arising in the hilar regions of the lungs were evident

No lesions were present in the spleen, lymph nodes or kidneys nor in the organs from the 10 untreated animals serving as controls No sarcoid-like epithelioid cell granulomas were noted in any of the rats

The significance of the lesions observed in the lungs and liver is not clear Pulmonary embolization invariably occurs after lymphangiography in man (LEE 1967, FISCHER 1969) DALION et coll (1965) have reported that part of the Lipiodol injected intravenously can be demonstrated in the lungs at 48 hours The fat of the Lipiodol molecules persisted for about one month whereas the iodine fraction was rapidly eliminated As the former was partly taken up by

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IRRADIATED CEREBELLAR MEDULLOBLASTOMA IN A MONOZYGOTIC TWIN

Growth, neurology and chromosomes 13 years after treatment

F BODOR, C H HÅKANSSON and M LINDGREN

A five-year old boy was irradiated for a medulloblastoma in 1960 at this department He was a monozygotic twin, and at an investigation of the effects on the chromosomes, following the irradiation of the primary tumour and the cerebrospinal canal, abnormalities in the chromosomes were ascertained (LINDGREN & NORRYD 1962)

The patient is now, 13 years later, still alive and at work, and it was therefore considered of interest to compare his development with that of his twin brother, and to investigate whether any chromosomal aberrations still remain

Report of the case Male born 1955 Monozygotic twin Weight at birth (first born) 3 050 g brother s weight 2 290 g Length at birth 49 cm, brother s length 46 cm

The patient was operated upon in 1960 for a medulloblastoma Between the ruptured tonsils a large bluish red tumour bulged forth This proved to be an off shoot of a tumour at least the size of a hen s egg
the vermis and
scopically the
The main part
the right cereb ... bottom of the fourth ventricle A Torkildsen s shunt was not carried out and the dura was left open

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REFERENCES

BAGLEY C. M. JR., ROTH J. A., THOMAS L. B. and DEVITA V. T. JR. Liver biopsy in Hodgkin's disease. Ann. intern. Med. 76 (1972), 219.

BRINCKER H. Epitheloid cell granulomas in Hodgkin's disease. Acta path. microbiol. scand. 78 (1970), 19.

DALTON J., GUERBET M. et DELAVILLE A. Destinée du lipodiol injectée par voie intraveineuse ou lymphatique. *Thérapie 20 (1965). 789.*

FISCHER H. W. Complications of lymphography. *In*: Recent results in cancer research, vol. 23, p. 24. Springer Verlag, Berlin—Heidelberg—New York 1969.

GEORGI M. Kontrastanfärbung der Leber. Eine seltene Komplikation während der Lymphographie. Fortschr. Röntgenstr. 106 (1967), 853.

GILLY G. und TAENZER V. Kontrastmittelspeicherung in der Leber nach Lymphographie. Fortschr. Röntgenstr. 106 (1967), 888.

HALLGRIMSON J. and CLOUSE M. E. Pulmonary oil emboli after lymphography. Arch. Path. 80 (1965), 426.

HECHT H., BERDORN W. and BAKER D. Hepatic oil embolization following lymphangiography in a child with neuroblastoma. Amer. J. Roentgenol. 104 (1968), 860.

KADIN M. E., DONALDSON S. S. and DORFMAN R. F. Isolated granulomas in Hodgkin's disease. New Engl. J. Med. 283 (1970), 859.

LEE B. J. Evaluation of the patient with lymphoma by means of lymphangiography: comments on importance and complications. Lymphangiography in Hodgkin's disease: indications and contraindications. *In*: Progress in lymphology, p. 127. Georg Thieme Verlag, Stuttgart 1967.

OEHLERT W. und DISCHLER W. Unspezifische Mesenchymreaktion und Parenchymschaden der Leber bei Patienten mit Morbus Hodgkin. Dtsch. med. Wschr. 97 (1972), 323.

PERSSON B. and NAVERSTEN Y. Technetium 99m sulfide colloid preparation *for scintigraphy* of the reticuloendothelial system. Acta radiol. Ther. Phys. Biol. 9 (1970), 567.

WALLACE S. Case report of patient who died subsequent to lymphangiography. *In*: Progress in lymphology, p. 135. Georg Thieme Verlag, Stuttgart 1967.

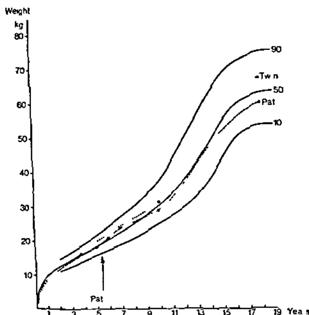


Fig 2 Differences in weight of patient and his twin brother while growing up The 10 50 and 90 percentiles are plotted in

Chromosomal investigations

For technical details about the chromosome preparation in 1960, see LINDGREN & NORRYD

Present examination The chromosomes were prepared from short term leucocyte cultures according to the technique of MOORHEAD et coll (1960), with some modifications Mitoses were arrested in metaphase by addition of Vinblastin (0 005 μg per ml medium) 1 h before fixation After 30 min in sodium citrate 1 % followed fixation in methanol acetic acid (3 1) The preparations were then dried in air in the conventional way without heating or flaming Staining was performed with Giemsa stain 2 ml diluted in 100 ml phosphate buffer at pH 7 0 100 cells were photographed and 50 of these karyotyped The frequency of polyploidy was calculated on repeated scanning of the preparations The number of polyploid cells was recorded percentually on the basis of 1 000 cells

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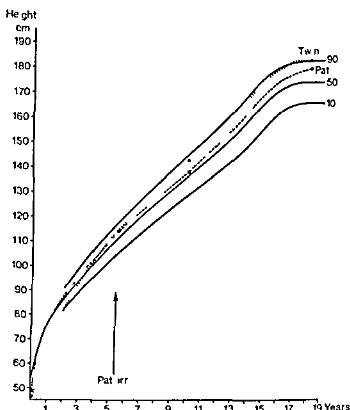


Fig 1 Differences in height of patient and his twin brother while growing up The 10 50 and 90 percentiles of the normal population are plotted in

Irradiation The patient received irradiation (180 kV HVL 0 93 mm Cu) towards three occipital ports of entry (10 cm × 6 cm field) Both sides of the target volume were first treated alternately with a dose to the skin of 200 R (= 120 rad tumour dose) Attempts to increase the dose caused nausea The lowest daily dose was 50 R to the skin (= 30 rad tumour dose) As the patient s nausea proved to be temporary it was possible to increase the dose and when the tumour had been given approximately 1 200 rad treatment of the middle occipital field was started with 200 R (= 100 rad tumour dose) only one of the occipital fields was irradiated each day (6 days a week) The entire occipital target volume was finally treated and a dose of 4 000 to 4 500 rad to the tumour area was given during 44 days

Following the occipital treatment the spinal canal was irradiated Two fields on each side immediately lateral to the spinal processes were used an upper measuring 18 cm × 5 cm and a lower 19 cm × 5 cm with an oblique beam direction of 40° and 35° respectively The irradiation was given in two series at an interval of 58 days and with a total (absorbed) dose of 1 500 rad/79 days The time interval was necessitated by a reduction of the white blood cells The first analysis of the chromosomes was performed four days after the first series of irradiation when the spine had received a dose of 1 100 rad/14 days

Following the irradiation at this department the patient has not undergone irradiation or any roentgen examination except mass screening carried out by the public health service

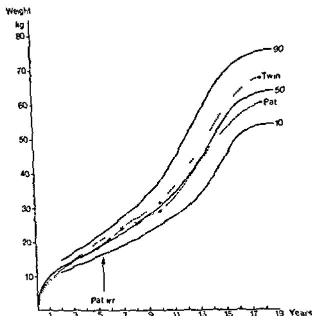


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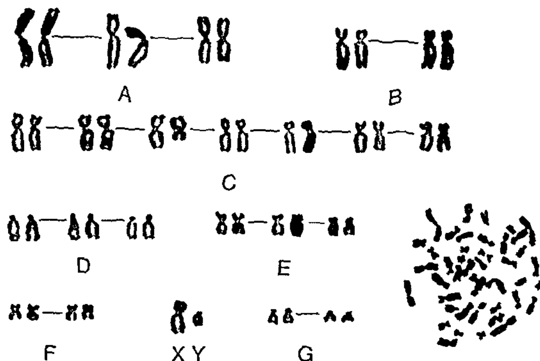


Fig. 3. Karyogram with a deletion in group C

Staining method. G-banding was performed according to a modification of the method of SEABRIGHT (1972). The slides were immersed for 20 s in a mixture of equal parts of trypsin solution 0.25 % and versene solution 0.02 %. After rinsing in a balanced salt solution, the slides were stained for 5 min in Giemsa stain (1 part Giemsas Lösung, Merck, 50 parts 0.01 M phosphate buffer, pH = 7.0), rinsed in running water and air dried.

Data acquisition. The Giemsa-stained-banded chromosome No. 1 with a break on a short arm (Fig. 4 c) was used for the presentation according to the principle of FLEISCHMANN et coll. (1971). A Zeiss Photomikroskop, Ultraphot II, and Copex Orto Rapid film were used. The negative was enlarged and transferred to a positive image up to 60 mm of length of chromosome No. 1 and the film scanned by a microdensitometer. The sampling interval was 0.5 mm and the chromosome quantitated and digitized into a matrix having 120 × 120 elements. The aperture of the phototube at the film plane was 0.5 mm and the response linear.

Computation. Univac 1108 was used for the computation. The method of smoothing has been worked out by GUSTAFSSON & TODD-POKROPEK (to be published). A contour map in the form of isobrightness curves was used with 20

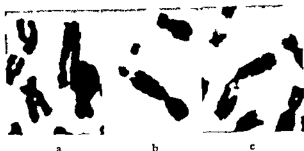


Fig 4 Unstable abnormalities a) Acentric fragment b) Minutes c) Chromatide break

density levels of the gray scale of the photographed chromosome (Fig 5) and a three axis presentation (isometric display)

Coloured chromosome The estimation of the density of the bands of the chromosomes will be easier by a conversion of the isobrightness to colour in a scale from low density (blue) to high density (red) The method of colouring has been developed at the Faculty of Technology, Lund (BERGSTROM & HERTZ (1972) Fig 7 illustrates a chromosome treated by this method Using red, blue, and yellow all colours can be presented and in this case the density scale is divided into 6 % steps In Fig 4 c there is a defect which may appear to be caused by a loss of substance However, the additional information in Figs 5, 6 and 7 displays not only the discontinuity in one of the short arm chromatides, but also that the part of the chromatide peripheral to the centromere contains a higher elevated area in the isometric display (Fig 6), corresponding to more closely assembled isobrightness curves in Fig 5 This may be interpreted as a collection of the stained material of DNA into a contraction, and the total mass of stained nuclein acid can be assumed to be the same as in the other, non broken chromatide

The concept of gaps and breaks of chromatides will be treated in a separate paper

Results

The patient and his twin brother have been followed up at regular intervals during the years after irradiation It may now be valuable to compare their development and to consider the condition of the patient, particularly in regard to the following points (1) The increase in height and weight as compared with that of his twin brother, (2) the mental and neurologic condition (3) remaining chromosomal aberrations in the peripheral blood denoting development of lines from damaged stem cells, (4) signs of leucaemic induction



Fig. 5

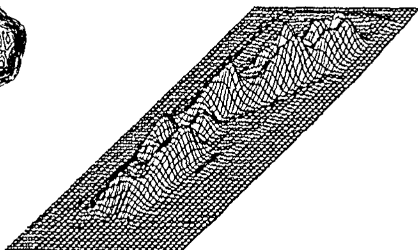


Fig. 6

Fig. 5. Chromosome No. 1 with a break in the short arm. (Rotated 180°.)
Fig. 6. The chromosome from Fig. 5 in three dimensional appearance.

Height. The patient was somewhat longer than his brother at birth but when he fell ill at five years of age he was as tall as his brother. Following treatment, his height was slightly reduced in comparison with his brother's, and this reduction proved to be permanent (Fig. 1). The height of the twins was also related to that of a normal population. The variable values for the twins have been plotted on diagrams as drawn up by TANNER & WHITEHOUSE (1966) and 10, 50, and 90 percentiles were plotted in. Both brothers lie within the 90 % confidence interval. The difference in height is thus not significant but the slight inhibition of the patient's growth in height may be due to the irradiation.

The treated boy has no reduced working capacity, as far as we have been able to ascertain. The twins share a job in a factory, each working 14 days at a time, after which the other twin relieves his brother, who then works 14 days on his father's farm.

Weight. The patient weighed more than his brother at birth, and his weight increased parallel to his brother's until the time of irradiation. Thereafter, the increase in weight was slightly less than his brother's. At ten years of age there was a difference of 2.5 kg (Fig. 2, based on the same principle as Fig. 1). These

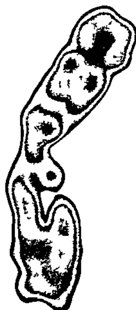




Fig 7 Chromosome No 1 with a break in the short arm as presented by the colour technique (Rotated 180°)

Table 1

Chromosome numbers of irradiated twin in 1960 and 1973

	1960	1973
Total cells	113	100
2 n	95 (84 %)	96 (96 %)
2 n ≠ 46	18 (16 %)	4 (4 %)

Table 2

Numbers of structurally normal and abnormal cells in 1960 and in 1973

	1960	1973
No structural abnormalities	71 %	79 %
D centric chromosomes	14 %	–
Other structural abnormalities	15 %	21 %

values lie on both sides of the rate of growth for a normal population The difference is now, at 18 years of age, 4 kg, i e the patient's weight is 5 % lower than his brother's

Neurology At the time when he was irradiated he was mentally alert for his age He presented choked discs of 1 to 1 5 D, nystagmus bilaterally of cerebellar type and slight ocular motor paresis to the right and general ataxia At follow-ups, evident improvement has been ascertained A slight ataxia on the right side was revealed by the nose finger test at the most recent examination (1973) The neurologic condition of the patient was otherwise excellent, with no indication of any mental retardation

Chromosomes 1960 The healthy brother had a normal karyotype (50 meta phases examined) The irradiated brother had a chromosomal number variation from 45 to 52, and 18 out of 113 cells (16 %) deviated from number 46 Abnormal chromosomes were found in 33 out of 113 cells (29 %) One or more dicentrics were found in 16 cells, and 17 had other deviations such as acentrics and new chromosomal types About 29 % of the leucocytes exhibited the results of one or more chromosomal breaks Different deviations were often present in the same cell The incidence of polyploidy was 0 6 % in the healthy twin and about 10 times higher in the patient (5 3 %) 54 % of these cells had 1 to 6 dicentrics

Table 3

White blood cells

Year	WBC	N	E	B	L	M	Comment
1960	2 800	67	2	—	29	1	at irr
1961	5 700	42	12	—	38	8	8 months later
1965	6 100	60	—	—	40	—	
1966	5 700	75	1	—	23	1	
1967	4 100	56	3	1	35	5	
1973	4 200	39	1	—	48	12	

1973. The numerical and structural abnormalities of the chromosomes are presented and compared with the results from 1960 in Tables 1 and 2.

As to the *numerical aberrations* (Table 1) 4 out of 100 cells deviated from the diploid chromosome number. Each of the aneuploid metaphase plates had 45 chromosomes. The numerical abnormalities affected different chromosomal groups in the karyotypes (groups A, B, C, and G). The range of the chromosomal numerical variation in the earlier examination (1960) was from 45 to 52, and the number of aneuploid cells was four times higher than in 1973. Seven out of 1 000 screened cells were polyploid. This value is lower (0.7 %) than it was in 1960 (5.3 %).

Various forms of chromosomal structural aberrations, such as breaks, deletions, minutes, gaps, pericentric inversions and translocations, were found in 21 % out of photographed and analyzed cells (Figs. 3, 4). Different structural abnormalities could often be detected in the same cell. Dicentrics, ring-chromosome types and new chromosome types were not present. One or more various types of break were found in 8 % of all cells. Minutes, gaps and acentric fragments occurred to a small degree. 11 % of the cells had unstable abnormalities. Deletions, translocations and pericentric inversions were present to the same extent with slight deviations. 7 metaphases from 50 karyotyped cells contained such 'stable' abnormalities (15 %). The quantity of 'stable' cells was 10 %.

A stable aberration means an aberration which may be persisting in a cell clone. Stable cells have only stable aberrations. Unstable abnormalities are liable to be lost or to undergo further alteration at cell division. These new concepts were introduced by BUCKTON et coll. (1962).

White blood cells. The cell count in the peripheral blood from the beginning of the treatment in 1960 until 1973 is shown in Table 3, thus, no signs of leucemogenesis were evident.

Discussion

Inhibited development of growing bones as a result of irradiation is a common phenomenon met with in radiation therapy ARKIN et coll (1950) were the first to describe a scoliosis in a young patient who had been treated for a Wilms' tumour Several reports have been published since then on inhibited growth of bone due to irradiation in children and young persons BERDON et coll (1965) described growth disturbances in children treated for Wilms' tumour and neuroblastoma The degree to which the spine was affected depended on the age of the patient and the dose received It is important that 'retarded, distorted and abnormal bone growth resulted regardless of whether ortho- or supervoltage therapy was used The exact doses to the vertebrae are not given, but the tumour dose was within 2 000 to 3 300 rad, which permits the assumption that at least 40 to 50 per cent of the volume of the vertebrae received the same dose

BORDZILOWSKA et coll (1972) observed changes in the spine following irradiation in ten out of eleven children who had received radiation therapy for Wilms tumour The dose was 3 000 rad or more in all of the ten cases, but was lower in the eleventh, being only 2 000 rad

The present case received 1 100 rad in 11 days at the age of five years The oldest patient of BORDZILOWSKA et coll was only two years and three months old, all of their cases were thus considerably younger than our patient

The present case does not contradict the assertion of BERDON et coll that bones in a physiologically more active state are more sensitive to radiation than bones which are growing more slowly The changes in growth (height as well as weight) were slightly retarded in comparison with the twin brother

Chromosomal aberrations Chromosomal injury due to therapeutic irradiation was first reported by TOUGH et coll (1960) Several reports on persistent chromosomal aberrations following irradiation have been published since then (BENDER & GOOCH 1962 1963, BUCKTON et coll DEKABAN 1965, COURT BROWN et coll 1967)

In the present case, different structural deviations were found in 21 per cent of the cells in 1973, whereas this figure was 29 per cent in 1960

Dicentric and ring chromosomes were not observed at the late examination, and the amount of breaks had decreased by 8 per cent The chromosomal picture 13 years ago was mainly characterized by 'unstable' deviations, but in 1973 the number of such aberrations was found to be lower Stable' cells were observed in 10 per cent, but no clone with typical deviation was discovered Irradiation is thus in this case not regarded as responsible for a new stem line Several authors have calculated the presence of unstable and stable aberrations as a function of time after irradiation BUCKTON et coll investigated 58 patients with ankylosing spondylitis who had received radiation treatment of the spine The pa

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RÉSUMÉ

Les auteurs ont reexamine au point de vue de la croissance, de l aspect neurologique et chromosomique, une paire de jumeaux monozygotes treize ans apres que l un des jumeaux eut éte irradie pour medulloblastome cerebelleux Sa taille et son poids etaient un peu inferieures à celles de son jumeau mais ses courbes etaient dans les limites d une population normale Son état neurologique etait presque completement redevenu normal Les auteurs ont trouve des aberrations chromosomiques dans les lymphocytes en 1973 ils ont trouve 8 pour cent de cassures chromosomiques au lieu de 29 pour cent en 1960 Il n y avait pas de dicentriques alors que le taux de cette aberration etait de 14 pour cent en 1960 Il y avait des cellules stables dans 10 pour cent des cas mais les auteurs n ont par trouve des lignees de nouvelle souche

REFERENCES

ARKIN A M PACK G T RAUSHOFF N S and SIMON N Radiation induced scoliosis case report J Bone Jt Surg 32 A (1950), 401

BENDER M A and GOOCH P C. Persistent chromosome aberrations in irradiated human subjects Radiat Res 16 (1962), 44

— — Persistent chromosome aberrations in irradiated human subjects, II Three and one half year investigation Radiat Res 18 (1963), 389

BERDON W E, BAKER D H and BOYER J Unusual benign and malignant sequelae to childhood Amer J Roentgenol 93 (1965) 545

BERGSTROM L A och HERTZ H Datorer ritar färgbilder (In Swedish) Forskning och Framsteg 8 (1972) 15

BORDZILOWSKA I, STOBA C SULOCKA J Late radiation injury to the skeletal system following radiotherapy for Wilms tumor (In Polish) Pediat Pol 47 (1972), 1517

BUCKTON K E JAKOBS P E COURT BROWN W M and DOLL R A study of the chromosome damage persisting after X ray therapy for ankylosing spondylitis Lancet 1972 II, p 676

DEKABAN A S Persisting clone of cells with an abnormal chromosome in a woman previously irradiated J nucl Med 6 (1965), 740

FITZGERALD P H Radiation chromosomes and man N Z med J 73 (1971), 147

FLEISCHMANN T, GUSTAFSSON T and HÅKANSSON C H Computer-display of the chromosomal fluorescence patterns Hered tas 68 (1971) 325

GUSTAFSSON T and TODD POKROPEK A Filter with variable shape in radio-isotope image processing To be published

LINDGREN M and NORRYD C Chromosome aberrations in one of a pair of identical twins after roentgen irradiation of

MOORHEAD P S NOWELL P D
Chromosome preparations
Cell Res 20 (1960) 613 Exp

SEABRIGHT M The
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TOUGH I M BUCKTON K E, BAIBLE A G and COURT BROWN W M X ray induced chromosome damage in man Lancet 1960 II, p 849

tients were examined immediately before therapy, and were then followed up in some cases for as long as 20 years. Aberrations were also found at that time, and the unstable aberrations discovered declined by about 50 per cent during the first twelve months, while the stable aberrations still existed at the same percentage up to 20 years after treatment. The unstable aberrations had then declined to about 7 per cent. FITZGERALD (1971) emphasizes the importance of these findings. Chromosomal aberrations induced by irradiation can thus be detected many years after treatment. Furthermore, the early decline of the unstable aberrations is clearly demonstrated, as is the unchanged existence of the stable aberrations. According to his theory, the small lymphocytes have a long life span, and stable aberrations are therefore able to survive for decades, provided they do not become the target of a sensitizing antigen, as they have immunologic capacity. Signs of leukemia in the appearance of the white blood cells are not confirmed. A slight increase was noted in the total content of the white cells, with a moderate increase in the lymphocyte percentage, but without evident pathologic signs (Table 3).

SUMMARY

A pair of monozygotic twins has been reexamined with regard to growth, neurology and chromosomal appearance 13 years after one of the twins had been irradiated for cerebellar medulloblastoma. The height and weight were slightly reduced compared with the twin but the curves lie within the limit of a normal population. Almost complete restitution of the patient's neurologic signs has taken place. Chromosome aberrations were found in the lymphocytes: in 1973 8 per cent of breaks were found instead of 29 per cent in 1960. Dicentrics were not present while the value of this aberration was 14 per cent in 1960. Stable cells occurred in 10 per cent but no new stem lines were discovered.

ZUSAMMENFASSUNG

Ein Paar monozygoter Zwillinge ist 13 Jahre nach der Bestrahlung des einen der Zwillinge wegen eines cerebellaren Medulloblastoms hinsichtlich des Wachstums, des neurologischen und chromosomalen Bildes nachuntersucht worden. Die Grösse und das Gewicht waren etwas vermindert verglichen mit dem Zwilling, die Kurven liegen jedoch innerhalb der Grenze einer Normalpopulation. Es hatte eine nahezu vollständige Restitution der neurologischen Befunde des Patienten stattgefunden. Chromosomale Veränderungen wurden in den Lymphozyten gefunden: 1973 fand man 8 Prozent Brüche anstatt 29 Prozent 1960. Dizentrische Chromosomen waren nicht vorhanden gegenüber einem Wert von 14 Prozent 1960. Stabile Zellformen fand man in 10 Prozent, neue Stammlinien sind jedoch nicht gefunden worden.

chromatic attenuation technique is not suitable because the presence of adipose tissue and intestinal gas in the path of the radiation gives uncontrollable variations in transmission It is also in practice difficult to provide constant thickness of absorber, e g by immersion of the patient in water These limitations can be partially eliminated by using two photon energies, one of which is so low that the attenuation is highly dependent upon the atomic number of the absorber while the other is so high that the attenuation is mainly dependent upon the mass of absorber (in units of g cm^{-2})

In a method called 'X ray spectrophotometry' (JACOBSON 1964, 1970) a roentgen tube with a special high potential generator and a filtering unit for generating two narrow energy bands is used The radiation beam passes through the patient and two servo controlled measuring wedges with attenuation pro perties corresponding to bone mineral and soft tissue respectively The transmitted photons are registered with a scintillation detector The wedges are automatically kept in such a position that the radiation fluxes at the scintillation detector are constant When the object being examined is placed in the path of the radiation beam the wedges are automatically withdrawn and the displacement is a quantitative measure of the amount of analysed substances (bone mineral and soft tissue) in the irradiated volume of the object Clinical applications and an investigation of various measurement sites have recently been presented (DALEN 1973)

The dichromatic attenuation technique using two photon energies from one or two isotopes was first described for determinations with the two photon energies alternating in the same stationary geometry (REED & ATKINSON 1965, REED 1966, JUDY 1970) This technique has been applied to appendicular parts of the skeleton e g femur and phalanges

In the first usable method for measuring bone minerals in the axial skeleton with the dichromatic attenuation technique the isotopes ^{241}Am (59 6 keV) and ^{137}Cs (662 keV) are utilized as well as a scanning procedure in which the radiation beam moves transversally over the site of measurement The two photon energies form a common radiation beam and are detected simultaneously with a scintillation detector (ROOS et coll 1970, 1971) This method has also been applied with the isotope ^{153}Gd which emits photons with energies around 44 and 100 keV (MAZESS et coll 1970, JUDY et coll 1971, 1972)

Theory

The possibility of separating bone mineral from soft tissue when measuring two transmitted photon energies depends upon the values of the attenuation coefficients and the thickness of the attenuating substances Photoelectric

FROM THE RADIATION PHYSICS DEPARTMENT (DIRECTOR: ASSOC. PROF. H. SKOLDBORN), SAHLGRENSKA SJUKHUSET, UNIVERSITY OF GOTHENBURG, S-413 45 GOTHENBURG, SWEDEN.

DUAL PHOTON ABSORPTIOMETRY IN LUMBAR VERTEBRAE

I. Theory and method

B O ROOS and H. SKOLDBORN

During recent years the bone mineral content has begun to be determined using gamma radiation from radioactive isotopes and counting transmitted photons with a scintillation detector and relevant electronics The most *usual method is the monochromatic attenuation technique using one photon* energy from an isotope, usually ^{125}I (mainly 27 4 keV) or ^{241}Am (59 6 keV). The radiation beam can either be moved at right angles to the length of the bone being examined (CAMERON et coll 1962, CAMERON & SORENSON 1963, SORENSON & CAMERON 1967) or be used for stationary measurement (NILSSON 1966) In both cases a reference measurement is required in which the radiation beam passes through a soft tissue bolus of the same thickness as the part of the body in question In practice this condition is most easily fulfilled when measuring bones of the extremities (e g radius and ulna, phalanges, condyle of femur, calcaneus)

For measuring transmission in parts of the skeleton in the trunk the mono-

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During recent years the bone mineral content has begun to be determined using gamma radiation from radioactive isotopes and counting transmitted photons with a scintillation detector and relevant electronics The most usual method is the monochromatic attenuation technique using one photon energy from an isotope, usually ^{125}I (mainly 27 4 keV) or ^{241}Am (59 6 keV) The radiation beam can either be moved at right angles to the length of the bone being examined (CAMERON et coll 1962, CAMERON & SORENSON 1963, SORENSON & CAMERON 1967) or be used for stationary measurement (NILSSON 1966) In both cases a reference measurement is required in which the radiation beam passes through a soft tissue bolus of the same thickness as the part of the body in question In practice this condition is most easily fulfilled when measuring bones of the extremities (e g radius and ulna, phalanges, condyle of femur, calcaneus)

For measuring transmission in parts of the skeleton in the trunk the mono-

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passage through a patient or a phantom The law of exponential attenuation ($R = R_0 e^{-\mu m}$) will then give the following if the radiation beam is assumed to pass all three types of tissue

$$R = R_0 e^{-\mu_S m_S - \mu_B m_B - \mu_F m_F} \tag{1}$$

$$R' = R'_0 e^{-\mu'_S m_S - \mu'_B m_B - \mu'_F m_F}$$

This equation system is solved with reference to m_B

$$m_B = \frac{\mu'_S \ln(R_0/R) - \mu_S \ln(R'_0/R') - (\mu'_S \mu_F - \mu_S \mu'_F)\, m_F}{\mu'_S \mu_B - \mu_S \mu'_B} \tag{2}$$

Equation (2) can be expressed more clearly by introducing some further designations

$$D_{SB} = \mu'_S \mu_B - \mu_S \mu'_B$$

$$D_{SF} = \mu'_S \mu_F - \mu_S \mu'_F$$

$$N = \mu'_S \ln(R_0/R) - \mu_S \ln(R'_0/R') \tag{3}$$

Substitution of these expressions in equation (2) then gives

$$m_B = \frac{N - D_{SF}\, m_F}{D_{SB}} \tag{4}$$

With knowledge of μ_S and μ'_S and measurement of the count numbers R_0, R, R'_0 and R' a numerical value for N can be calculated by measurement of transmission with two photon energies D_{SF} and D_{SB} may be regarded as constant for a certain measurement geometry and can be determined by calibration The amount of bone tissue [illegible] equation (4) with some uncertainty [illegible] the path of the radiation In order [illegible] variable is required, either registration with three different photon energies or exact determination of the path length of the radiation through the absorber (the thickness of the patient at the site of measurement) The latter method is difficult when measuring through the trunk since gas in the d[illegible]ive tract [illegible] method [illegible] point of [illegible] measurements on either side of the vertebral column (where $m_B = 0$)

Energy channels Compton scattering Both photon energies are registered [illegible]

[illegible] range [illegible] keV while the other records radiation from [137]Cs in the

absorption gives increased attenuation at low photon energies (below approximately 100 keV) in heavy substances such as bone salts, while the compton effect gives an attenuation purely proportional to density above approximately 140 keV. The ratio between the mass attenuation coefficients for bone mineral and soft tissue increases when the photon energy decreases Thus, the lower the photon energy used, the more selective the absorption in the bone tissue This is however counteracted by the fact that transmission must be sufficient to provide a statistically acceptable count rate even when examining parts of the skeleton with thick soft tissue coverings For the lower photon energy the theoretical optimum when measuring through the trunk seems to be of the order of 35 to 45 keV (WOOTEN 1971) but the somewhat higher energy of 59 6 keV may be selected without disadvantage for the following reasons (1) the absorption in adipose tissue differs less from the absorption in lean soft tissue, (2) the transmitted photon flux varies less with thickness of the soft tissue For the higher photon energy the optimum is constant within the range 200–800 keV (WOOTEN 1971) Thus, any energy within these ranges may be selected

The selection of ^{241}Am (59 6 keV) and ^{137}Cs (662 keV) has partially been based on factors other than absorption-statistical aspects Both the photon energies are, for example, pure, which means that there is no interference from any nearby photon energy which can give a varying attenuation coefficient depending on the thickness of the object Furthermore, both isotopes have long half lives and are commercially available with the required activity and safely enclosed in capsules.

Designations The following designations and units will be used

m the amount of absorber in the path of the radiation beam, g cm^{-2}
B index for bone tissue
S index for lean soft tissue
F index for adipose tissue
μ mass attenuation coefficient, cm^2 g^{-1}
P index for transmission measurement through a special perspex phantom
R count number when the radiation beam has passed an absorber
R_0 count number with the absorber removed

Magnitudes concerning the photon energy 662 keV are indicated by the prime sign while magnitudes concerning the photon energy 59 6 keV are shown without the prime sign A number of other designations will be introduced in the following section but these are more easily explained in their context

Mathematical theory We assume that radiation quality and radiation geometry are such that both the photon energies are exponentially attenuated during

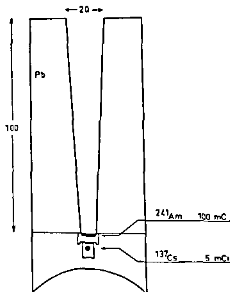


Fig 2 ^{241}Am and ^{137}Cs sealed sources in lead container with conical radiation outlet The ^{137}Cs radiation is only slightly attenuated in the ^{241}Am source capsule Distances in mm

patients R_0 and R'_0 are related to R_P and R'_P with two experimentally determined proportionality factors, designated f and f′ respectively N is then calculated from the following final formula

$$N = \mu_S \ln \frac{f(R_P - kR'_P)}{R - kR} - \mu_S \ln \frac{f R'_P}{R'} \qquad (6)$$

Apparatus

Mechanics The set up of the radiation sources, patient and detector is illustrated in Fig 1 The patient lies on his back with his legs bent so that the vertebral column is parallel with the couch The distance between the radiation sources and the scintillation crystal is 530 mm The couch is mounted on ball bearings and may be moved transversally by means of a screw and a reversible motor On the screw there is a cam disc with pegs which activate a micro-switch coupled to an electronic control unit This enables stepwise transport of the couch in steps of 2, 4 or 8 mm Stationary transmission measurement occurs during the pauses between the steps

Radiation sources and radiation geometry The radiation sources consist of 100 mCi ^{241}Am (Amersham code AMC 6, active surface circular with diameter

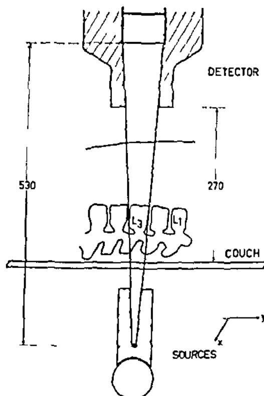


Fig 1 Arrangement of sources, lead collimation and detector The lumbar vertebrae of a patient in the measuring position on the couch are indicated The x direction is transversal, the y direction longitudinal Distances in mm

energy range 550 to 800 keV For accurate determination it is essential that the detector and other electronic equipment are stable and allowed to warm up for a sufficiently long time (several hours) before measurements are made.

A certain fraction k of the radiation from the ^{137}Cs source undergoes such compton scattering in the patient and detector that it will be registered in the energy channel for ^{241}Am Measurements with the ^{241}Am source removed and with different thicknesses of absorber have shown that the compton fraction k for the given measurement geometry and with the energy channels specified above has a value of 0 045 relatively independent of the thickness of the patient

If this interference is subtracted from the count number in the ^{241}Am channel equation (3) will have the following appearance

$$N = \mu' s \ln \frac{R_0}{R-kR'} - \mu s \ln \frac{R'_0}{R} \tag{5}$$

Count rates without absorber R_0 and R'_0, the number of counts without the absorber, cannot be measured directly due to dead time losses An indirect procedure is applied in which the radiation through a cylindrical perspex phantom (R_P and R'_P) is registered in connection with measurements in

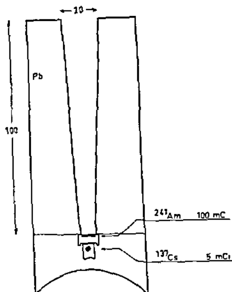


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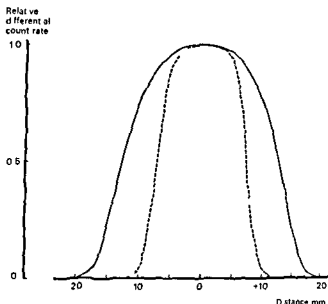


Fig 3 Beam profiles for circular (—) and rectangular (- - -) geometry measured in the transverse direction

7 2 mm) and 5 mCi ^{137}Cs (Amersham code CDC 804, active diameter 3 mm) The sources are mounted together in a lead shield so that the ^{241}Am source is supported by the ^{137}Cs source, the photon radiation of which thus passes through the ^{241}Am source The two radiation beams have common collimations in line with the collimator of the detector (Fig 2)

The cross section of the radiation beam is circular The beam is divergent and has a geometrical diameter of 35 mm at a height of 90 mm above the couch In certain cases a narrower field of radiation may be desirable It is possible to mount lead linings in the capsule and in the collimator of the detector With these linings the geometrical extent of the radiation beam in *the transversal direction is reduced to 18 mm (at 90 mm height)* while the extension in the longitudinal direction remains unchanged—rectangular geometry

Profile curves for the two radiation geometries have been measured with stepwise transmission measurement through a water phantom containing a lead disc with straight edges at right angle to the direction of movement The lead disc was at a height of 90 mm above the couch The results are presented in Fig 3 For each radiation geometry the profile curves for ^{241}Am and ^{137}Cs are fairly similar and their mean values are presented in Fig 3 The half value breadth for circular geometry is 25 4 mm and for rectangular geometry 14 4 mm The profile curve for the rectangular radiation geometry shows that it is better defined than the circular geometry When measuring over a vertebra according to the scanning principle the rectangular geometry will be pre-

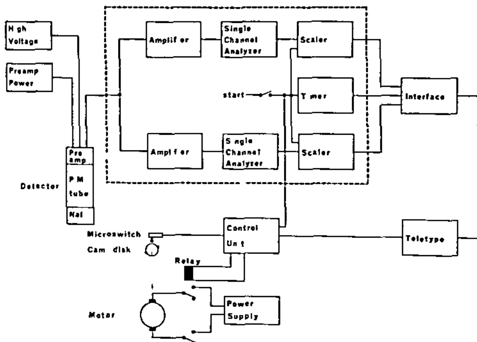


Fig 4 Block diagram of detector dual channel analyzer and the automatic step-scanning system

ferable because the measurement points involving bone will be easier to demarcate in the result

Detector and electronics The detector consists of a NaI(Tl)-crystal (7 5 cm diameter × 5 cm high), photomultiplier tube (RCA 8054) and preamplifier. The high potential to the detector is obtained from a stable high potential supply (Oltronix LS529R) The counts from the detector are analysed and counted in two separate channels (Picker Twinscaler) Registration occurs numerically and on punched tape with Teletype Model 33 The electronics also comprise a motor and automatic control device for stepwise shift of the couch After each shift the counts from the detector are counted simultaneously in the two energy channels during a predetermined period of time The electronic set-up is illustrated in the block diagram in Fig 4

Dead time The influence of dead time losses on count rates has been analysed with aluminium discs as the absorber and simultaneous counting in the two channels In a semilogarithmic diagram (Fig 5) a linear relationship was ob-

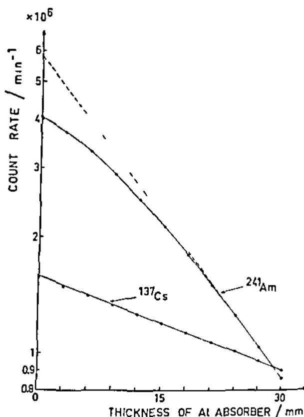


Fig 5 Count rates measured simultaneously in both energy channels with varying thickness of aluminium absorber, indicating negligible dead time losses for count rates below 10^6 min^{-1}

tained between the count rate and thickness of the absorber for count rates below 10^6 min^{-1} The count rate seldom exceeds 10^6 min^{-1} when measuring in patients and the dead time losses can therefore normally be neglected and the count numbers obtained be used uncorrected

Data processing The primary result of patient measurement is a punched tape containing all count numbers measured in sequence and count numbers for stationary measurement through a perspex phantom, R_P and R'_P

The calculation of N according to equation (6) is done with a minicomputer type PDP8/E, a plot of N as a function of the measurement position also being obtained (Fig 6) The investigation comprises 41 measurement points The four columns contain R and R′, the primary count numbers, K, which are N multiplied by 10^4 in order to obtain more convenient figures, and T, an interval figure for plotting K

The right hand part of Fig 6 shows the automatic plot of K at the various points along the measurement path The scale is not correct in the x-direction due to the line feed mechanism of the Teletype

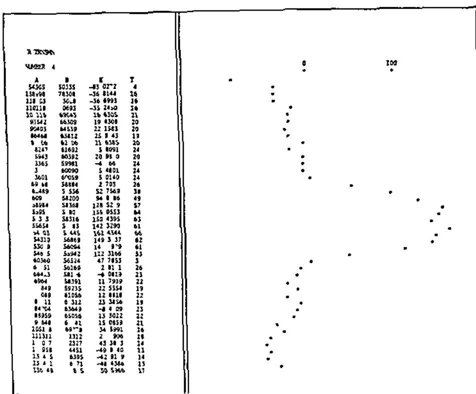


Fig 6 Print out of result from the PDP8/E computer Left Primary counts A (^{241}Am) and B (^{137}Cs) calculated K values (K = N 10^4) and T an interval number for the K plot Right K plot on the Teletype note the number of measuring points on both sides of the vertebra

Method of patient measurement

The centre of the relevant vertebral body (usually L3) is marked on the abdomen during fluoroscopy with a diagnostic roentgen apparatus, the patient lying on his back with his legs bent Transmission measurement is then performed with the patient lying in the same position and the central axis of the radiation beam directed at the mark on the abdomen by means of indicator lamps on the detector (Fig 7) Side supports for the knees make it easier for the patient to lie still during measurement No other fixation devices are required

The measurement path has a length of 160 mm, giving 41 measurement points at intervals of 4 mm With a measurement time of 0 25 min for each point the total time for measurement becomes just over 10 min In addition

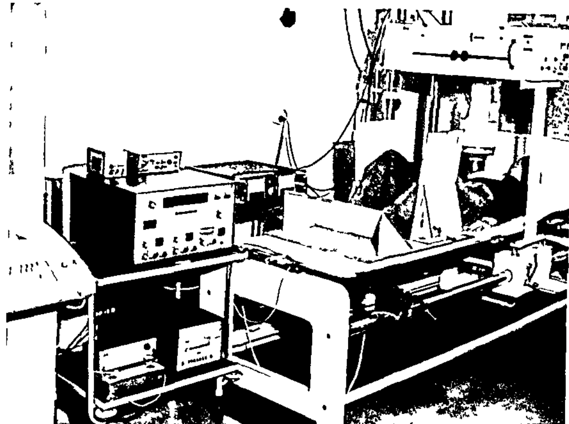


Fig 7 Measuring apparatus and electronics Patient lying supine in the measuring position with the legs bent and leaning against a wooden support

there is the time for transport and writing, which makes the total examination time approximately 15 min per measurement path An examination often consists of 3 measurement paths, one through the mark and the other two on either side and at 5 mm distance from it

Evaluation of patient data Fig 8 illustrates the N values from a patient measurement as a function of the transversal measurement position x (for interpretation of the curve see equation 4) The length of the measurement path is such that the radiation beam is free from bone tissue at several points on either side of the vertebra Attenuation is thus dependent solely upon the composition of the patient regarding soft tissue and adipose tissue If $m_B = 0$ in equation (4), then $N = D_{SF}m_F$ The mass attenuation coefficient for adipose tissue is less than that for water at 59 6 keV, i e D_{SF} is less than zero The occurrence of adipose tissue in the path of the radiation beam at these points thus gives negative values of N If the radiation only passes through soft tissue N will be equal to zero independently of the thickness of the absorber

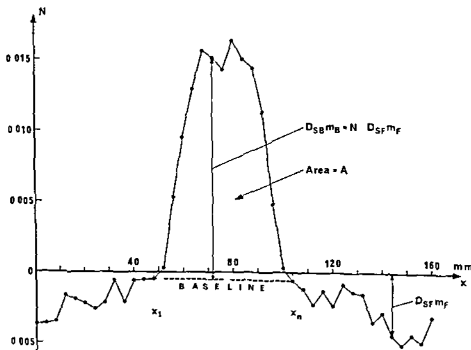


Fig 8 A plot of the same patient data as in Fig 6 The influence of adipose tissue, $D_{SF}m_F$ placement of baseline and summation area A is illustrated

The presence of bone tissue in the path of the radiation beam gives N a positive increase, $D_{SB}m_B$ (c f Fig 8) The magnitude of the adipose tissue contribution $D_{SF}m_F$ at these points is unknown, however We approximate as follows Points number 1 and n (Fig 8) are assumed to be the points lying nearest the vertebra and yet free from bone tissue Between these points the adipose tissue contribution is interpolated to N with a straight line, the base line At each point between 1 and n $D_{SB}m_B$ is then assumed to be equal to the height of the point over the base line

Integration of the N curve over the base line gives the area A, which is related to the amount of bone mineral as follows

$$A = \int_{x_1}^{x_n} D_{SB}m_B \, dx = D_{SB} \int_{x_1}^{x_n} m_B \, dx = D_{SB}M_B \qquad (7)$$

where M_B in the unit g cm^{-1} is the integrated amount of bone mineral along the measurement path M_B may be regarded as the amount of bone mineral

in a 10 mm *transverse section* through the patient, given as the mean value over the effective extent of the radiation beam, approximately 25 mm, in the longitudinal direction.

In practice, N is drawn as a polygon through the measurement points (Fig. 8) and the area A calculated as follows:

$$A = \left[\sum_{i=2}^{n-1} N_i - \frac{n-2}{2}(N_1+N_n) \right] \cdot \Delta x \tag{8}$$

The results of the examination are then stated by M_B as follows:

$$M_B = \frac{A}{D_{xB}} \tag{9}$$

Discussion

Precision. The statistical variation in the count rates gives an error of precision at each measurement point. The standard deviation in N (according to equation 3) increases with increasing mass attenuation coefficient but decreases with increasing count number. In a normal patient examination with count numbers around 50 000 in both channels the standard deviation in N is approximately 0.001. *The integrated area A (Fig. 8) is normally 0.05 to 0.09 (in cm³ g⁻¹).* If all measurement points gave errors of precision with the same sign and of the same magnitude as the standard deviation this would give an error of the order of 7—10 per cent in the area A and thus also in the result M_B. In reality, the signs of the errors of precision vary randomly and on adding the area A they tend partially to cancel out one another.

Reproducibility. The reproducibility during short periods of time has been tested by repeated measurements on both phantoms and patients. The measurement series have comprised at least 10 measurements with a maximum of 2 measurements being performed on the same day. The standard deviation in these series is approximately 3 per cent in phantom measurements and varies from 3 to 8 per cent in patient measurements. The increased spread in patient values is due to difficulties in reproducing the exact measurement position (the measurement path over the centre of the *vertebral body) and the difficulty* for the patient in lying completely still during the measurement. These sources of error might probably be reduced, however, with improved technique.

To summarize, the method described enables measurement of variations with time in the bone mineral content in deeply situated bones such as vertebrae with a precision exceeding 10 per cent. The result is expressed in g cm⁻¹

but this value is based upon calibration measurements of phantoms and must be regarded as a relative measure of the amount of bone mineral The main sources of error affecting the absolute precision, apart from the statistical variations, are the content of adipose tissue in the vertebra itself and in the vertebral canal, geometrical errors due to the irregular form of the vertebra and the profile of the radiation beam and errors in constants The total effect of these sources of error can be investigated by measurement of vertebrae in vitro before and after ashing

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SUMMARY

An objective method measuring the bone mineral content of a vertebra is described Photons transmitted from ^{241}Am and ^{137}Cs are recorded simultaneously with a scintillation detector Registration occurs at a number of equidistant points along a transversal measurement path The result is independent of the thickness of soft tissue intestinal gas and a constant thickness of subcutaneous adipose tissue The reproducibility during patient measurements exceeds 10 per cent The method is well suited for repeat examinations of skeletal mass in the vertebral column

ZUSAMMENFASSUNG

Es wird eine objektive Methode den Knochen Mineralgehalt der Wirbel zu messen beschrieben Transmittierte Photonen von ^{241}Am und ^{137}Cs werden gleichzeitig mit einem Szintillationsdetektor registriert Die Registrierung geschieht simultan in einer Anzahl von Punkten gleichen Abstands langs einer transversalen Messtrecke Das Messergebnis ist unabhangig von der Dicke der weichen Gewebe des intestinalen Gases und einer konstanten Dicke des subkutanen Fettgewebes Die Reproduzierbarkeit wahrend der Messung von Patienten uberschritt 10 Prozent Die Methode ist gut anwendbar fur wiederholte Untersuchungen der Skelettmasse der Wirbelsaule

RÉSUMÉ

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[illegible] vertébrale

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des tissus mous des [illegible]
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REFERENCES

CAMERON J R and SORENSON J Measurement of bone mineral in vivo An improved method Science 142 (1963), 230

— GRANT R and MACGREGOR R An improved technic for the measurement of bone mineral content in vivo Radiology 78 (1962), 117

DALÉN N Bone mineral assay Measuring sites, clinical applications Thesis Department of Medical Engineering, Karolinska Institutet, Stockholm 1973

JACOBSON B *X ray spectrophotometry in vivo Amer J Roentgenol 91 (1964), 202*

— Bone salt determinations by X ray spectrophotometry Bone Measurement Conf, Chicago, Ill (1970) Proc CONF-700515, p 237

JUDY P F A dichromatic attenuation technique for the in vivo determination of bone mineral content, Ph D Thesis, University of Wisconsin Madison Wisconsin, 1970

— ORT M G, KIANIAN K and MAZESS R B Developments in the dichromatic attenuation technique for the determination of bone mineral content in vivo USAEC Progress Report COO 1422 97 (1971)

— *CAMERON J R, JONES K M and ORT M G Determination of vertebral bone mineral* mass by transmission measurements USAEC Progress Report COO 1422 126 (1972)

MAZESS R B, ORT M JUDY P and MATHER W Absorptiometric bone mineral determination using ^{153}Gd Bone Measurement Conf, Chicago, Ill (1970), Proc CONF 700515 p 308

NILSSON B E R Post traumatic osteopenia A quantitative study of the bone mineral mass in the femur following fracture of the tibia in man using americium 241 as a photon source Acta orthop scand (1966) Suppl No 91

REED G W The assessment of bone mineralisation from the relative transmission of Am 241 and Cs-137 radiations Phys in Med Biol 11 (1966) 174 (abstract)

— and ATKINSON P J Studies of bone mineralisation Brit Emp Cancer Campaign Annual Rep 43 (1965), 465

ROOS B ROSENGREN B and SKOLDBORN H Determination of bone mineral content in lumbar vertebrae by a double gamma ray technique Bone Measurement Conference, Chicago, Ill (1970), Proc CONF-700515 p 243

— — — Die Bestimmung des Mineralgehaltes der Lendenwirbel durch eine doppelte Gamma Strahlentechnik Rontgen Bl 24 (1971) 190

SORENSON J A and CAMERON J R A reliable in vivo measurement of bone mineral content J Bone Jt Surg 49A (1967), 481

WOOTEN W W Bone densitometry The two photon technique and fat content of the bone Thesis University of California, Los Angeles 1971

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SECONDARY ANAEMIA IN MALIGNANCY

Prognostic significance of haematologic changes six years after irradiation of cervical carcinoma

NINA EINHORN and P REIZENSTEIN

In anaemia secondary to cancer, the serum folic acid and iron concentrations are reduced and the *erythrocyte sedimentation rate* is increased (RAO et coll 1963, HELLMAN et coll 1964, HOOGSTRAATEN et coll 1965, EINHORN & REIZENSTEIN 1966) It has also been shown that in cases of carcinoma of the uterine cervix the degree of anaemia is significantly correlated to the serum *folate* clearance rate, the serum iron, and the ESR, and that one year after radiation therapy neither the anaemia nor the ESR, neither the serum iron nor the serum folate had improved significantly (EINHORN & REIZENSTEIN 1971) The present report evaluates the *same parameters* and their possible prognostic significance in the same patients as in the previous report, now after an observation time of *five* years *after* the primary irradiation

Material and Methods The material consists of 41 patients examined before initial irradiation, 36 of these were also examined one year after therapy At

From the Department of Gynecology (Director Ass *Professor* B Tjernberg), Radium Hemmet and the Section of Haematology, Department of Medicine (Director Prof H Lagerlof Karolinska sjukhuset S-104 01 Stockholm Sweden *Submitted* for publication 5 *February* 1974

Table 1

Differences between failures and cured patients

Haematologic variable	Before irradiation				One year after irradiation			
	Failures		Cures		Failures		Cures	
	No of pat	M±SE	No of pat	M±SE	No of pat	M±SE	No of pat	M±SE
ESR, mm/h	*9*	*23±5*	*32*	*18±2*	*8*	*31±4*	*25*	*18±2*
Serum iron, mg/100 ml	9	0.12±0.02	32	0.12±0.07	9	0.09±0.01	27	0.09±0.01
Serum folate, ng/ml	9	4.1±0.7	32	4.0±0.7	9	4.3±0.8	27	5.0±0.6
Haemoglobin, per cent of 13.6 g/100 ml	9	83±3	32	80±1	9	80±2	28	80±2

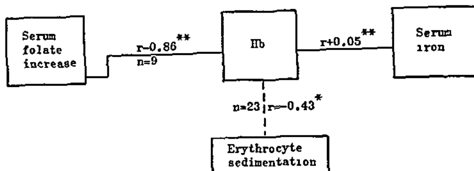
this time all 41 patients were alive, although five were not examined for different technical reasons. Four to six years after irradiation only 32 patients were alive, 25 of these were examined (seven patients had left Stockholm and could not be examined haematologically). The results of haematologic and clinical examinations up to one year after treatment have been reported previously (EINHORN & REIZENSTEIN).

The patients were seen regularly for a clinical follow-up once every two months during the first year after treatment and later, if symptom-free, with time

Table 2

Haematologic values in cured patients before and after irradiation. The significance of the differences was calculated as compared to the values before irradiation. Standard error of the mean is indicated

Haematologic variable	Before irradiation		One year after irradiation			4–6 years after irradiation		
	No of pat	Result of tests	No of pat	Result of tests	Signif of diff	No of pat	Result of tests	Signif of diff
ESR, mm/h	32	18±2	25	18±2		25	18±2	
Serum iron mg/100 ml	32	0.121±0.007	27	0.089±0.006	**	23	0.100±0.006	*
Serum folate, ng/ml	32	4.01±0.7	27	5.0±0.6		25	5.7±0.5	*
Haemoglobin per cent of 13.6 g/100 ml	32	80±1	25	80±2		24	89±3	**



Correlation between anaemia folates and iron one year after treatment in failures (———) and in cured patients ( ) Increase value before minus one year after the treatment r cor relation coefficient n — number of patients asterisk — degree of statistical significance

intervals increasing up to twice a year during the 6th year after treatment Although the seven patients who left Stockholm could not attend the haematologic follow-up, no patient was lost from the clinical follow up Nine patients died of recurrence of the tumour during the follow up time, these patients are called failures, the remaining cases surviving for at least 4 to 6 years were free from recognizable tumour recurrence and are called cures No patient died from intercurrent disease

The method used in the treatment of these patients has been described previously (KOTTMEIER 1964, EINHORN & REIZENSTEIN 1971) Statistical analyses of correlations between the variables were performed by the computer program of ZACHRISSON & REIZENSTEIN (1968) The level of significance between different mean values is indicated in the tables as follows $0.05 > p > 0.01$ by *, $0.01 > p > 0.001$ by **, and $p < 0.001$ by *** The haemoglobin (Hb) was determined by the cyanomet haemoglobine method, 13 6 g/100 ml being used as the 100 % value Serum iron (SeFe) was measured colorimetrically by the o-phenon trolin method The serum folic acid activity (SFAA) was bio-assayed with l casei (REIZENSTEIN 1965) The ESR was measured after one hour

Results

Haematologic values before treatment In retrospect, no significant differences were found in the initial haematologic values between the 32 cured patients and the nine failures (Table 1)

Table 3

Change in the haematologic values after irradiation. The values are given as the difference between post-treatment minus pre-treatment values and the standard errors of the mean of the differences are indicated

Haematologic variable	Failures		Cures			
	1 year after irradiation		1 year after irradiation		4–6 years after irradiation	
	No of pat	Result of tests	No of pat	Result of tests	No of pat	Result of tests
ESR mm/h	9	+6±7	24	+1±2	25	+2±3
Serum iron mg/100 ml	9	−2.9±2.2	27	−0.032±0.009**	23	0.016±0.009
Serum folate ng/ml	9	+2.4±4.8	27	−0.7±1.0	25	+2.3±0.4***
Haemoglobin per cent of 13.6 g/100 ml	9	−2.8±2.3	27	+1.3±1.5	25	−9.7±3.5**

Haematologic values after one year. No significant improvement in the haematologic values one year after treatment occurred in either group. In fact, in the patients who later appeared cured, the serum iron had dropped significantly one year after therapy. Numerically, this drop was observed also in the failures but was not significant (Table 3).

The failures had a significantly ($p < 0.01$) higher ESR and a numerically, but not significantly lower serum folate than those who later appeared cured (Table 1). The more anaemic the failures were, the lower their serum folates and serum iron (Figure). These correlations were statistically significant. No similar correlation was found in the cured patients.

Haematologic values after 4 to 6 years. The patients who were cured improved the Hb and the serum folates as compared to the values before treatment ($p < 0.01 < 0.05$, respectively) and also to the values one year after treatment (Table 2). The serum iron was higher than after one year but still significantly lower ($0.05 > p > 0.01$) than before treatment (Table 2).

Discussion

Even in relatively early cases of malignant tumours a reduction in the serum folic acid activity (EINHORN & REIZENSTEIN 1966) and in the serum iron (HEVESEY & KOTTMEIER 1960) as well as an increase in the plasma elimination of folic acid (EINHORN & REIZENSTEIN 1971) and of iron (HEVESEY & KOTTMEIER) has been found.

Pre treatment haematologic values had no prognostic significance Mean serum iron values decreased after irradiation also if the prognosis was favourable

Persistently pathologic ESR and low serum folate values after treatment may be signs of a poor prognosis However, this applies to the mean values and conclusions regarding individual patients must be drawn with caution Statistically significant improvements may occur although the general effects of cervical carcinoma are so small that mean haemoglobins, folates, and irons are all within normal limits, both before and after treatment

Surprisingly enough, the folates and the anaemia had not improved significantly even in the cured patients one year after treatment, although they had improved after 4 to 6 years Different hypothetic explanations are possible It has been demonstrated that tissue injury and inflammation cause the same folate, iron and haemoglobin changes as in the present material, and that such changes are correlated to an increased reticuloendothelial uptake of folate, iron and erythrocytes (ELMAN et coll 1970, WIKLUND-HAMMARSTROM et coll 1971, ÅSEN et coll 1971, REIZENSTEIN & GHEORGHESCU 1974) The present absence of improvement of anaemia, folates, ESR, and iron one year after treatment even in patients with a favourable prognosis could be explained by persistent inflammatory tissue changes, caused by the irradiation, this inflammation may have decreased after 4 to 6 years when the haematologic values are normalized, if the patient remains free from recurrence

Acknowledgement

This investigation was supported by the Cancer Society of Stockholm

SUMMARY

Haemoglobin serum folate, iron values and ESR were determined in cases of carcinoma of the uterine cervix before one year and 4 to 6 years after irradiation Nine of the 41 examined patients died of their tumours Their pre treatment values did not differ significantly from those in patients with a favourable prognosis but the ESR and the serum folate values were more pathologic after one year than the values in patients with more favourable prognoses Twenty five patients were followed for 4 to 6 years The haematologic values improved significantly only after 4 to 6 years possibly because of long lasting radiation induced local tissue reaction Persistently pathologic post treatment serum iron values had no prognostic significance

ZUSAMMENFASSUNG

Es wurden Hamoglobin Serum Folat Eisenwerte und ESR bei Fallen eines Cervix Karzinoms des Uterus vor, ein Jahr und 4 bis 6 Jahre nach Bestrahlung bestimmt Neun der 41 untersuchten Patienten starben an ihren Tumoren Deren Werte vor der Behandlung

unterschieden sich nicht signifikant von denen derjenigen Patienten mit einer günstigen Prognose, jedoch waren die ESR und Serum Folat Werte mehr pathologisch nach einem Jahr als die Werte von Patienten mit günstigerer Prognose Vierundzwanzig Patienten wurden 4 bis 6 Jahre lang verfolgt. Die hämatologischen Werte waren nur nach 4 bis 6 Jahren signifikant verbessert, möglicherweise wegen der langhaltenden Strahlen-hervorgerufenen lokalen Gewebsreaktionen Bestehende pathologische Serumeisenwerte nach der Behandlung hatten keine prognostische Bedeutung

RÉSUMÉ

Les taux d'hémoglobine, de folates sériques la sidérémie et la vitesse de sédimentation ont été déterminés dans des cas de cancer du col de l'utérus avant, un an et de quatre à six ans après l'irradiation Neuf des 41 malades examinées sont mortes de leurs tumeurs Les résultats de ces examens avant le traitement ne différaient pas significativement chez ces malades de ceux des malades qui ont eu un pronostic favorable, mais la vitesse de sédimentation et le taux de folates sériques étaient plus anormaux au bout d'un an que les valeurs chez les malades qui avaient un pronostic plus favorable Vingt-cinq malades ont été suivies de 4 à 6 ans Les résultats de leurs examens hématologiques ne se sont améliorés significativement qu'après 4 à 6 ans, peut-être à cause de la réaction tissulaire locale de longue durée induite par les radiations Des valeurs de sidérémie restant anormales après le traitement n'ont pas eu de signification pronostique

REFERENCES

ÅSEN P, LILJEDAHL S-O and REIZENSTEIN P Studies of trauma III Plasma iron elimination rate Acta chir scand 135 (1971), 275

EINHORN J and REIZENSTEIN P Metabolic studies on folic acid in malignancy, Cancer 26 (1966), 310

EINHORN N and REIZENSTEIN P Secondary anemia in malignancy Folic acid in cervical carcinoma In Radiobiologic investigations Acta radiol (1971) Suppl No 310, p 75

ELMAN A, EINHORN J, OLHAGEN B and REIZENSTEIN P Metabolic studies of folic acid in non-malignant diseases Acta med scand 187 (1970), 347

HELLMAN S, IANNOTTI A T and BERTINO J R Determinations of the levels of serum folate in patients with carcinoma of head and neck treated with Methotrexate Cancer 24 (1964), 105

HEVESY G-C and KOTTMEIER H-L Turnover of plasma iron in cancer patients prior to and after treatment Acta obstet gynec scand 39 (1960), 675

HOOGSTRAATEN B, BAKER H and GILBERT H S Serum folate and serum vitamin B_{12} in patients with malignant hematologic diseases Cancer 25 (1965), 1933

KOTTMEIER H-L Surgical and radiation treatment of carcinoma of the uterine cervix Acta obstet gynec scand 43 (1964), 1

— Problems relating to classification and stage grouping of malignant tumours in the female pelvis In Krebs-Dokumentation und Statistik maligner Tumoren, p 15 Bericht der 10 Jahrestagung des "Arbeitsausschuss Medizin", Berlin, Oktober 1965 Schattauer-Verlag, Stuttgart 1966

RAO R P B, LAGERLÖF B, EINHORN J and REIZENSTEIN P Low serum folic acid in malignancy Lancet 1963 I, p 1193

— Folic acid activity in leukemia and cancer Cancer 25 (1965), 221
REIZENSTEIN P Errors and artefacts in serum folic acid assays Acta med scand 178 (1965), 133
— and GHEORGHESCU B Secondary anemia (XV) and reticuloendothelial uptake in cancer Acta haemat, 51 (1974), 9
WIKLUND HAMMARSTROM B, GHEORGHESCU B, LILJEDAHL S O, NYLIND B and REIZENSTEIN P Reticuloendothelial activity and some metabolic changes related to trauma *In* The reticuloendothelial system and immune phenomena, p 333 Edited by N R DiLuzio and K Flemming Plenum, New York 1971
ZACHRISSON L and REIZENSTEIN P Mapping of correlations between clinical variables Int Congr Medicine, p 168, Warzaw 1968

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BLADDER AND INTESTINAL INJURIES FOLLOWING INTRACAVITARY IRRADIATION OF CARCINOMA OF THE UTERINE CERVIX

L. G. FRIBERG and J. E. JOHNSSON

Radiation treatment of carcinoma of the cervix uteri is sometimes accompanied by complications from surrounding organs, especially the bladder and intestines. A series of investigations has been carried out to clarify the factors in the treatment technique responsible for these sequelae (FLETCHER et coll. 1958, 1962, FLETCHER 1971, KOTTMEIER 1964, STROCKBINE et coll. 1970, JOELSSON et coll. 1971, VAN DER WALL 1971). The intention of the present work has been to indicate the role played by the position of the intracavitary applicators in causing reactions to irradiation of the bladder and intestines.

Material and Methods. The material consisted of 83 patients with carcinoma of the cervix uteri Stage I. Two intracavitary treatments at an interval of three weeks have been given by the Stockholm technique. External roentgen radiation was also administered to two lower abdominal and two lower dorsal fields, directed towards the parametria and the pelvic wall. Factors: 180 kV, HVL

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1 mm Cu, SSD 60 cm Absorbed surface dose 500 rad × 3 to each field, field size about 200 cm² with one field treated per treatment day The abdominal fields were usually dealt with in connection with the first intracavitary treatment, and the dorsal fields at the time of the second treatment The material was divided into two groups according to the technique used for the intracavitary treatment The same intrauterine and vaginal applicators were utilized in both these series

Group I consisted of 30 patients treated in 1968 The intrauterine and vaginal applicators were fixed to each other in an ideal position by a locking arrangement, i e the intrauterine applicator was placed orthogonally in direct contact with and at the centre of the principal plane of the vaginal applicator (JOHNSSON & NORDBERG 1973), this guaranteed a well-defined distribution of dose around the applicators The total dose at Point A (2 cm laterally and 2 cm cranially to the intrauterine applicator's point of contact with the vaginal applicator at about the external os of the cervix was 6 500 rad (see Fig 1 in JOHNSSON & NORDBERG)

Group II consisted of 53 patients treated during the years 1952, 1953 and 1958 The intrauterine and vaginal applicators were placed in the uterus and vagina, respectively, without being fixed Due to the varying interpositions of the applicators, the distribution of dose around them could not later be calculated The theoretic dose at Point A was about 6 500 rad (JOHNSSON & NORDBERG 1973) Extraperitoneal lymphadenectomy was performed four months after completion of the radiation treatment in 33 cases of the group (GORTON 1953), the two groups are thus not strictly comparable

Reaction to irradiation in the bladder and the intestines have been registered in both groups, and the period of observation has extended to four years following completion of treatment Grading of the radiation reactions has been done according to the following (GRAY & KOTTMEIER 1957) scale

Bladder reaction Grade I—Minimal objective changes with mild symptoms Grade II—More or less extensive necrosis on cystoscopy or the patient suffered from pain or haemorrhage which required repeated blood transfusions and even admission to hospital Grade III—Radiation fistulas

Rectal reaction Grade I—Mild or no symptoms with minimal changes in the mucosa Grade II—Frequent painful stools and passage of mucus with blood for many months, even for years Areas of necrosis, ulceration or moderate stenosis evident Grade III—Rectal stenosis severe enough to require colostomy Grade IV—Radiation fistulas

Table

Incidence of bladder and intestinal reaction

	No of patients	Bladder reaction grade		Intestinal reaction grade	
		II	III	II	III
Group I					
Applicators fixed to each other	30	1	0	1	0
Group II					
Applicators fixed	53	11	0	9	2

Results

One patient in group I experienced bleeding from the bladder for 2 to 3 weeks Cystoscopy revealed mild oedema in the trigone area and a bleeding telangiectasis No ulceration or necrosis were observed The blood vessel was coagulated since when the patient has had no symptoms This reaction was judged as belonging to grades I—II Another patient had persistent rectal trouble in the form of periodically recurrent diarrhoea with some tenesmus Roentgen examination of the colon revealed slight to moderate stenosis of the distal sigmoid This reaction was considered as belonging to grades I—II (Table)

Eleven patients in group II had bladder reactions with ulceration or necrosis of the mucous membrane at cystoscopy (grade II) These have usually not been severe and all the patients were free from symptoms at the end of the observation period Rectal reactions were recorded in 11 patients in 2 of whom marked changes in the lower part of the rectum demanded colostomy (grade III) this could be closed in one of these patients after a year as the rectal lesion had healed The other patient had an obstruction of the small intestine two months after colostomy had been performed Laparotomy revealed a part of the small bowel firmly adherent to the uterus Side to side small bowel anastomosis was performed The rectal changes healed with stenosis of the rectum The other 9 patients had mild to moderate rectal reactions classified as grade II (Table) they were all free from symptoms at the end of the observation period

Discussion

Several reasons for undesirable reactions in tissues after treatment with ionizing radiation exist only those explainable by the positions of the intracavitary applicators will however be discussed Furthermore only those cases of



Fig 1 Barium enema Stricture in upper part of the rectum

a tumour at an early stage (stage I) have been selected in order to eliminate complications from the bladder and the intestine due to tumour necroses

The frequency of reactions in the bladder and the intestines was higher in group II than in group I The higher frequency of bladder reactions may to some extent be explained by the lymph adenectomies undertaken, as injury to the bladder and its vessels cannot be completely avoided with these measures There was however, no difference in the frequency of complication between the surgical and the non surgical patients The intestine and its vessels also lie in the vicinity of the area where surgery was performed, but the frequency of intestinal reaction was equally great for the surgical patients as for those who had not undergone operation FLETCHER et coll (1958) proved that surgical measures following irradiation treatment may cause an increase in complications from the bladder and the intestines

It is probable that the main reason for the higher frequency of reactions in the bladder and the intestines recorded in this group lies in the fact that the intra uterine and the vaginal applicators were not locked to each other and fixed The gauze packing of the vaginal applicator in those patients in whom the uterus lies anteflected presses against the portio, which pushes the uterus over into still more marked anteflection and may alter the position of the applicators in relation to one another (see Fig 3 in JOHNSSON & NORDBERG) This has been described by FLETCHER (1971) The use of gauze packing in patients already with a retroflected uterus causes it to tip over into more marked retroflection Even an inclination of 20° between the intrauterine and the vaginal applicator



Fig. 2. The intrauterine and vaginal applicator in situ locked to each other in ideal position.

causes the dose at a point 2 cm cranial to the intrauterine applicator's point of contact with the vaginal applicator, and 2 cm in the direction of inclination to be around 12 000 rad during the treatment time used (JOHNSSON & NORDBERG 1973). This point lies on a level with, or immediately cranially to, the area of the vesical trigone if the inclination be made with an anteflected uterus. It lies on a level with, or immediately above, the rear fornix in the rectum if the inclination be made with a retroflected uterus—localities where reactions to irradiation take place. Rectal reactions with the above localizations were recorded in 11 of 53 patients. This reaction was so marked in two of these patients that colostomy had to be performed.

In the material in which the applicators were locked to one another in a fixed 'ideal' position, thus avoiding unverifiable and high doses of irradiation, only one patient out of 30 developed a bladder reaction, one that proved to be of short duration. No patient had any reaction in the lower part of the rectum.

Intestinal reaction without objective findings in the lower part of the rectum was recorded in one patient in group I, the group in which the applicators had been locked together. One patient of group II had both a rectal reaction grade II, and a reaction in the small intestine. The reason for this may be explained as follows: the pressure of gauze packing on the vaginal applicator presses the uterus dorsally and cranially against the sacrum. Under mobile conditions, and especially if the uterus be small, so that the intrauterine applicator extends up into the fundus, those parts of the intestines lying in the true pelvis may receive extremely high doses of irradiation (FLETCHER 1971). The most common site for



Fig 3



Fig 4

Fig 3 Normal inferior mesenteric angiography

Fig 4 Inferior mesenteric angiography Complete occlusion of the superior haemorrhoidal artery and irregular lumen and occlusions in mural arteries in the rectum and sigmoid

this type of irradiation reaction is the area between the rectum and the sigmoid, although other parts of the intestines may also present such changes A large part of the small intestine often lies in the true pelvis Adhesions of the intestines to the genital organs are thought to be important factors in the development of intestinal reactions, and intestinal sections adhering to the uterus have been reported at operation in such cases (JOELSSON et coll 1971), our experience has, however, indicated that the latter have most often been caused by the irradiation

There are two types of reaction produced by the intrauterine applicator The first arises by the uterus or the fundus being pressed against the intestinal wall (for example of the rectum and sigmoid) If a sufficiently high dose has been given to the intestinal wall, necrosis may cover only a small area, be surrounded by oedema and, later, fibrosis resulting in stricture (Fig 1)

The localization of the intestinal changes in relation to the contours of the true pelvis, and the position of the intrauterine applicator, drawn in from roentgenograms obtained during an actual intracavitary treatment appear in



Fig 2 The intrauterine and vaginal applicator in situ locked to each other in ideal position

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application. It ought to be possible to reduce the frequency of this type of intestinal reaction by preventing the intrauterine applicator from reaching up into the uterine fundus.

Conclusion

The position of the intracavitary applicators in relation to one another has a decisive influence on the origin of bladder and intestinal reactions. Locking the intrauterine and the vaginal applicators to each other in a fixed position affords a *well definable distribution* of dose around them, and high and unverifiable doses in the bladder and the rectum may in this way be avoided. Injuries to the intestinal sections located in the upper part of the true pelvis (rectum, sigmoid and small intestine) are caused by the intrauterine applicator, and reduction in the risk of such injuries ought to be possible, partly through the use of shorter intrauterine applicators, and partly by changing their position in the true pelvis.

SUMMARY

Bladder and intestinal reactions have been investigated in two materials of carcinoma of the uterine cervix in intracavitary treatment with radium. In one the intrauterine and the vaginal applicators were locked together in a fixed position and in the other they were unlocked. The frequency of complications was lower in the former material. The reasons for the reaction in the bladder, rectum and intestinal sections lying in the true pelvis are discussed.

ZUSAMMENFASSUNG

Die Reaktionen der Blase und des Darms wurden bei zwei Gruppen eines Carcinoms der Cervix uteri bei intrakavitärer Radiumbehandlung untersucht. Bei der einen waren die intrauterinen und vaginalen Applikatoren in einer fixierten Lage miteinander verbunden, bei der anderen waren diese nicht miteinander verbunden. Die Komplikationsfrequenz war in der ersten Gruppe niedriger. Die Gründe für die Reaktionen der Blase, des Rektum und der intestinalen Abschnitte, die im kleinen Becken liegen, werden besprochen.

RÉSUMÉ

Les auteurs ont étudié les réactions vésicales et intestinales au traitement intra-cavitaire par le radium du cancer du col de l'utérus sur deux séries de malades. Dans une série les applicateurs intra utérins et vaginaux étaient solidarisés dans une position fixe et dans l'autre série ils étaient indépendants. La fréquence des complications était moindre dans la première série. Les auteurs examinent les raisons de la réaction vésicale, rectale et des parties de l'intestin qui sont situées dans le petit bassin.

application It ought to be possible to reduce the frequency of this type of intestinal reaction by preventing the intrauterine applicator from reaching up into the uterine fundus

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SUMMARY

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RÉSUMÉ

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REFERENCES

DENCKER H., H:SON HOLMDAHL K., LUNDERQUIST A., OLIVECRONA H. and TYLÉN U.: Mesenteric angiography in patients with radiation injury of the bowel after pelvis irradiation. Amer. J. Roentgenol. 114 (1972), 476.

FLETCHER G. H.: Cancer of the uterine cervix (Janeway Lecture, 1970). Amer. J. Roentgenol. *111 (1971), 225.*

— BROWN T. C. and RUTLEDGE F. N.: Clinical significance of rectal and bladder dose measurements in radium therapy of cancer of uterine cervix. Amer. J. Roentgenol. 79 (1958), 421.

— RUTLEDGE F. N. and CHAU P. M.: Policies of treatment in cancer of the cervix uteri. Amer. J. Roentgenol. 87 (1962), 2.

GRAY M. J. and KOTTMEIER H. L.: Rectal and bladder injuries following radium therapy for carcinoma of cervix at Radiumhemmet. Amer. J. Obstet. Gynec. 74 (1957), 1294.

GORTON G.: Post irradiative prophylactic extraperitoneal lymphadenectomy in carcinoma of the uterine cervix. Acta radiol. *(1953) Suppl. No. 100.*

JOELSSON I., RÄF L. and SÖDERBERG G.: Stenosis of small bowel as a complication in radiation therapy of carcinoma of the uterine cervix. Acta radiol. Ther. Phys. Biol. 10 (1971), 593.

JOHNSSON J. E. and NORDBERG U. B.: Dosimetric problems with radium in the intracavitary treatment of carcinoma of the uterine cervix. Acta radiol. Ther. Phys. Biol. 12 (1973), 257.

KOTTMEIER H. L.: Complications following radiation therapy in carcinoma of the cervix and their treatment. Amer. J. Obstet. Gynec. 88 (1964), 854.

STROCKBINE M. F., HANCOCK J. E. and FLETCHER G. H.: Complications in 831 patients with squamous cell carcinoma of the intact uterine cervix treated with 3 000 rads or more whole pelvis irradiation. Amer. J. Roentgenol. *108 (1970), 293.*

VAN DER WALT H.: Zur Problematik der Komplikationen nach Radiumbehandlung des Kollumkarzinom. Zbl. Gynäk. 93 (1971), 273.

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RESPONSE OF A MUSCULO-SKELETAL TUMOUR TO COMBINED RADIATION THERAPY

An in vitro/vivo investigation

DAVID S MUCKLE

Although it was first reported more than 60 years ago that elevated temperatures sensitised malignant tumours to irradiation, there has been little attempt to exploit this effect clinically or to carry out detailed investigations relating in vitro and in vivo tumour response Similarly, a beneficial synergistic action between

human cancer, and in 1913 he reported on 100 patients with advanced, histologically proven cancer of various sites (breast, bronchus, oesophagus and stomach) of these 100 tumours 32 regressed completely and 36 temporarily However the effectiveness of the combined treatment was evaluated on the basis of personal experience rather than on a comparative series of tumours treated by each therapy alone MÜLLER's publication was followed by several other reports, with a total consideration of 500 cases The conditions of treatment in each series were however too variable and the information often insufficient to permit a valid comparison of the published results (ARONS & SOKOLOFF 1937)

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WARREN (1935) treated 32 cases of advanced carcinoma and sarcoma in man with a combination of induced fever of 41 5° C and irradiation However, the remissions reported were usually of short duration (3 to 6 months) KORB (1939) showed a synergistic action between heat and irradiation on basal cell carcinomas, while WOEBER (1955) found that the effective radiation dose could be reduced in the presence of hyperthermia CRILE (1962) reported that the dose required to induce a regression in cutaneous metastases from a breast carcinoma was reduced from 1 500 rad to 600 rad if the skin was immersed in a water-bath at 44 to 46° C for 1 hour He also found that the squamous cell carcinoma, metastatic melanoma, and rectal carcinoma showed greater regression to radiation therapy *after* previous exposure to heat BARTH (1956) found that there was more skin injury after combined heat and irradiation than with irradiation alone, findings which were at variance with the results of BIRKNER & WACHSMANN (1949) and WOEBER (1955)

ROHDENBURG & PRIME (1921) using the Crocker 180 tumour in mice and spontaneous mammary tumours of the Lathrop mouse strain, and HILL (1934) using a Jensen sarcoma and an unidentified mouse tumour, all found regression of the malignant growth when subjected to heat plus a sub-lethal dose of irradiation ROHDENBURG & PRIME found no significant difference when either heat or radiation therapy was given first in combination therapy, while ARONS & SOKOLOFF (1937) and FUCHS (1935) reported that simultaneous therapy or the application of heat immediately subsequent to irradiation gave the best results JARES & WARREN (1937) found that a time interval of twelve to twenty-four hours between heat and irradiation reduced the beneficial effects of the combined therapy In a material of over 3 000 mice with Wood's sarcoma, the best results were obtained when hyperthermia was given first (49 5 per cent cure rate compared to 22 3 per cent)

The beneficial response of tumours to combined chemotherapy and radiation therapy has been reported in several series MANUILA et coll (1967) used a combination of nitrogen mustard and irradiation in Hodgkin's disease, while a synergistic effect between 5-fluorouracil and irradiation in pulmonary and oesophageal carcinoma has been reported (LOYE et coll 1960, ALLAIRE et coll 1961) The use of actinomycin D and irradiation in Wilms' tumour is another example of the adjuvant effect of combination therapy (FARBER et coll 1960)

In the present investigation the adjuvant effect of non-curative (sub optimal) irradiation in combination with a sub-optimal dose of local hyperthermia (tissue temperature above 42° C) or chemotherapy (Methotrexate) have been examined, and the results of in vitro measurements (respiration and anaerobic glycolysis) compared with the in vivo response (volumetric measurements and isotope uptake)

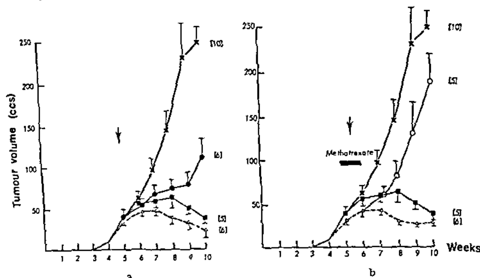


Fig 1 Changes in mean Vx2 tumour volume following various combination therapies The figures in brackets indicate the number of tumours (animals) at each point on the curves a) × untreated tumours ● hyperthermia ■ radiation therapy, △ hyperthermia + radiation therapy b) × untreated tumours, O methotrexate, ■ radiation therapy, △ radiation therapy + methotrexate

Materials and Methods

The experimental musculo-skeletal tumour used was the Vx2 tumour, a highly malignant growth which originated in a Shope virus-papilloma The tumour is characterized by rapid and predictable metastases to lymph nodes and lungs The tumour was passaged by periodic transfer of 1×10^{7} cells into the thigh muscles of New Zealand white rabbits, and the resulting tumour became palpable between 3 and 4 weeks later, the volume increased exponentially from 5 to 9 weeks, untreated rabbits died at 10 weeks Therapy was applied at day 35 after cell inoculation and tumour volume measurements were made at weekly intervals, allowances being made for the normal tissues of the thigh

For the in vitro experiments cells were obtained from treated and control tumours by enzymatic disaggregation with trypsin and DNase (MUCKLE & DICKSON 1971) which does not injure cell metabolism

The O_2 uptake (cell respiration) and CO_2 production (anaerobic glycolysis) were measured in Warburg flasks, with 5 to 10×10^{7} cells per flask, incubated at 37 5° C (For details see MUCKLE & DICKSON)

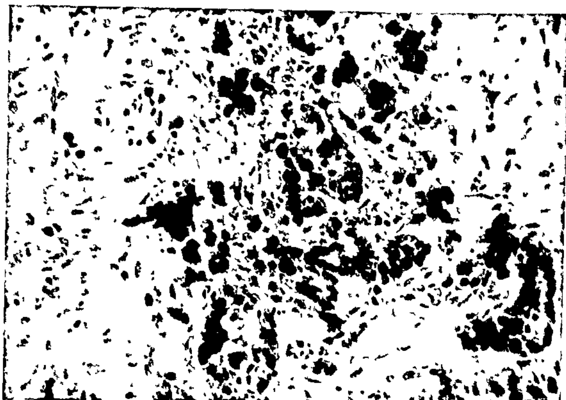


Fig 2 Untreated Vx2 tumour with ^{3}H thymidine uptake in groups of cells in a fibrous tissue stroma (×410)

For the in vivo treatment of tumours by heat or irradiation the rabbit was anaesthetized with intravenous Nembutal, 0 6 ml/kg Local hyperthermia was applied by immersion of the hind limb in a water-bath at 46° C, when an intratumour temperature of 42 to 43° C was reached, and maintained for 1 hour Needle electrodes measured tumour, thoracic, skin (ear) and abdominal temperatures

Radiation therapy, 1 000 rad TD, was applied by superficial radiation (140 kV, 8 mA, filtration 0 2 mm Cu and 1 0 mm Al) from 2 opposing fields, size 7 cm, FSD 25 cm Chemotherapy consisted of methotrexate given by 6 daily intravenous injections of 0 4 mg/kg into the ear vein, beginning on day 35 after tumour inoculation In combination therapy, irradiation was performed on day 38 or followed within 2 hours of hyperthermia

For isotope examinations 100 μCi ^{3}H-thymidine (thymidine methyl T, greater than 10 000 mCi/mM, Amersham) were injected into the femoral artery of the tumour bearing limb Biopsies were taken at regular intervals When combination therapy was given, the administration of the isotope followed immediately after the treatment ceased

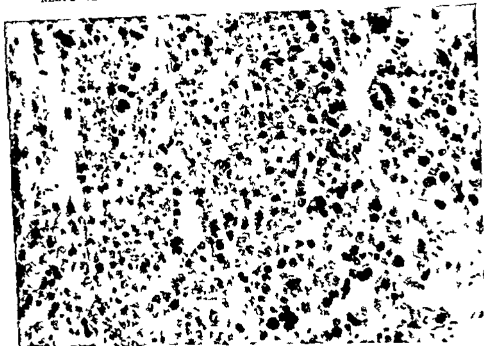


Fig 3 Treated Vx2 tumour (heat and irradiation) reduced ^{3}H thymidine 5 days after therapy Lysis and karyorrhexis in the Vx2 cells (×410)

Results

Fig 1 records the response of the primary tumour to hyperthermia and irradiation, alone or in combinatic
combination Each method,
tumour growth compared to
the control and treated tumours was significant at the 8th week ($p < 0.001$) However, the tumours treated by heat or methotrexate subsequently increased in volume, whereas the irradiated tumours decreased in size Following combination therapy, tumour volume continued to increase for a further 2 weeks Thereafter, the tumours given radiation therapy plus hyperthermia or plus methotrexate, decreased in volume, the tumour volume measurements for both types of dual therapy being comparable at each time point ($p > 0.05$), and the variability about the mean values being small The volumes of these tumours were significantly less than the corresponding volumes for tumours irradiated only ($p < 0.05$)

Table 1

Each Warburg flask contained 5–10 × 10⁶ Vx2 cells. The respiration (QO_2) and anaerobic glycolysis, (QCO_2) (in brackets) of Vx2 tumour cells following in vivo therapy. The figures quoted as a range were obtained from 3 separate tumours in each case. Q values represent µl gas exchanged/mg dry weight of cells/per hour

Therapy	24 hours	10 days	4 weeks
Irradiation (QO_2)	4.0–4.7	7.3–8.0	7.0–8.0
Irradiation (QCO_2)	(8.0–8.8)	(12.6–13.8)	(14.0–15.0)
Heat (42° C), 1 hour	2.5–3.3 (14.2–16.4)	4.5–5.6 (11.0–16.7)	6.5–8.1 (11.1–17.7)
Methotrexate	9.8–10.6 (14.4–14.6)	4.8–4.9 (8.8–10.0)	7.6–7.7 (14.0–16.0)
Untreated cells	7.7–9.2 (13.9–17.1)	7.5–9.2 (14.0–17.2)	7.3–8.9 (13.8–16.9)
Heat/irradiation	3.3–4.0 (7.0–8.1)	4.1–5.2 (9.0–10.0)	4.0–4.7 (14.5–16.3)
Irradiation/methotrexate	4.8–5.3 (8.6–9.2)	4.4–5.5 (8.8–9.8)	5.3–5.6 (12.2–13.6)

Fig. 2 depicts the normal histology of the Vx2 muscle tumour, and the isotope labelling is seen within the nests of Vx2 cells. The changes after hyperthermia and irradiation consist of loss of the normal tumour architecture, with cell lysis (Fig. 3). There is a peripheral rim of intact cells with isotope uptake. Viable Vx2 cells could be obtained from this area even when the central part was replaced by fibrous tissue 4 weeks after therapy. The potentiating effect of heat or methotrexate produced no striking alteration in the histologic picture following irradiation.

The Table records the respiration and anaerobic glycolysis of the treated tumours at 24 hours, 10 days and 4 weeks following therapy. Oxygen uptake and CO_2 production were linear over 4 hours in Warburg flasks, and for comparison the average QO_2 and QCO_2 values over this period have been used. Local heating for 1 hour caused a rapid decrease in respiration, with subsequent recovery of QO_2 values, while anaerobic glycolysis was unaffected. However, irradiation had a depressive effect on both respiration and glycolysis, measured 24 hours after therapy, but both these parameters recovered towards control values over the following 4 weeks. The effect of methotrexate was evident at 10 days as an *inhibition of both O_2 uptake and CO_2 production*, at 4 weeks these values returned to normal. The effects of combination therapy reflected a sum-

mation of the individual therapies, at 4 weeks there was synergism of action between irradiation and hyperthermia or methotrexate, indicated by persistent marked inhibition of respiration

The effects of combination therapy and each therapy alone on tumour volume, histology and isotope uptake (in vivo) were comparable to the effects on O_2 uptake, and to a lesser extent the CO_2 production (in vitro), after each form of treatment

Discussion

Experiments on the Vx2 musculo-skeletal tumour showed that the effects of irradiation were potentiated by heat or chemotherapy (Methotrexate) as assessed by a significant reduction in tumour volume (Fig 1) and the inhibition of respiration and anaerobic glycolysis (Table) Although the insensitivity of tumour growth measurements as the sole criterion in the assessment of cancer therapy is well recognized (STEEL & LAMERTON 1969), the effects of combination therapy were also shown by the marked reduction in radioactive ^{3}H thymidine uptake in treated tumours, and the presence of cell lysis and karyorrhexis However, the biochemical and histologic results indicated that after combination therapy considerable numbers of metabolically and mitotically active Vx2 cells remained in the primary tumour and the animal survival data corroborated these findings, combination therapy having little effect on host death (74 ± 5 days in the treated rabbits compared to 70 ± 6 days in the controls) Vx2 tumour regression has been observed after combined rad at on theran [illegible]
SON & JOHNSTON 1968) [illegible]
(1935) described similar [illegible]
therapy as found in the above experiments

CRILE (1970) has drawn attention to the possibility that a primary tumour may act as the antigenic source necessary to maintain immunity against metastases With tumour systems in mice the incidence of secondary growths was significantly increased when the foot carrying the tumour was amputated as when the tumour was destroyed by irradiation CRILE suggested that the cells killed by irradiation maintained their antigenicity and prolonged the animals' immunity

Elevation of temperature results in vasodilatation and increased tissue or gen-
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WILDERS [illegible] work it is also clear that the relationship between primary tumour and metastases requires consideration in therapy It may be that irradiation can be enhanced by prior heat application, as suggested

by many authors in the earlier part of this century, and that the effects of elevated temperatures on cancer cell metabolism (disruption of cell membranes, arrest of mitotic division in metaphase, and prolongation of the lag phase with inhibition of the log phase of cell growth; for details see MUCKLE & DICKSON 1971) will sensitise the neoplasm to irradiation. Total body hyperthermia techniques as advocated by HENDERSON & PETTIGREW (1971), may be of value in future combined therapy in human cancer.

The value of the present in vivo/vitro investigation was the demonstration of biochemical changes paralleling tumour growth measurements. Such techniques could be of use in human therapy although the difficulty in obtaining cancer cells from solid neoplasms is well recognized; and a complex, pains-taking procedure was needed to obtain viable Vx2 cells from the established tumour (MUCKLE & DICKSON 1971, MUCKLE 1972). However, such advances in cancer physiology will be necessary to monitor the response of malignant cells to radiation therapy, either alone or in combination with heat or chemotherapy.

Acknowledgement

I would like to acknowledge the encouragement and advice of Prof I D A Johnston, Department of Surgery, Newcastle, and Dr J A Dickson, whose cooperation on the project was invaluable, especially the interpretation of the biochemical investigations carried out in the Cancer Research Unit, Newcastle upon Tyne

SUMMARY

An experimental musculo-skeletal tumour, the Vx2 tumour in rabbits, proved to be a convenient model to investigate the effects of irradiation, either alone or in combination with heat or chemotherapy In vitro (cell respiration and glycolysis) and in vivo (tumour growth, isotope uptake) measurements were carried out on treated and untreated tumours Combination therapy proved to be more effective on all parameters than either therapy alone, and the in vitro response of tumour cells after treatment paralleled the in vivo response The implications of combination therapy in man is discussed, with a view to *sensitising cancer cells to irradiation and potentiating the immune response*

ZUSAMMENFASSUNG

Ein experimenteller Muskel-Skelett-Tumor, der Vx2 Tumor bei Kaninchen, wurde als ein geeignetes Modell befunden, um die Effekte von Strahlung, entweder alleine oder in Kombination mit Wärme oder Chemotherapie zu untersuchen In vitro (Zell-Atmung und Glykolysis) und in vivo (Tumorwachstum, Isotopaufnahme) Untersuchungen wurden an behandelten und nicht behandelten Tumoren vorgenommen Die Kombinationstherapie erwies sich bei allen Parametern als stärker wirksam als jegliche Behandlung für sich, und die in vitro Reaktion der Tumorzellen nach einer Behandlung folgte der in vivo Reaktion Die Folgerungen einer kombinierten Therapie beim Menschen im Hinblick auf die Sensibilisierung von Krebszellen gegen Bestrahlung und die Verstärkung der Immunantwort werden diskutiert

RÉSUMÉ

Une tumeur musculo-squelettique experimentale, la tumeur Vx2 des lapins, s est montree etre un modele convenable pour etudier les effets de l irradiation soit seule, soit associee à la chaleur ou à la chimiotherapie Les mesures in vitro (respiration cellulaire et glycolyse) et in vivo (croissance tumorale fixation isotopique) ont ete effectuees sur des tumeurs traitées et non traitees L association therapeutique s est montre plus efficace sur tous les parametres qu aucun traitement isole et la reponse des tumeurs cellulaires après traitement in vitro est parallele a la reponse in vivo L'auteur examine les consequences de cette association thérapeutique chez l homme dans le dessein de sensibiliser les cellules cancereuses a l irradiation et de potentialiser la reponse immunitaire

REFERENCES

ALLAIRE F J THIEME E T and KOPPT D R Cancer chemotherapy with 5 fluorouracil alone and in combination with X ray therapy Cancer Chemother Abstr 14 (1961), 59

ARONS I and SOKOLOFF B Combined roentgentherapy and ultrasound Amer J Surg 36 (1937) 533

BARTH G Radiology Book Abstract p 172 1956

BIRKNER R und WACHSMANN F Über die Kombination von Rontgenstrahlen und Kurz-

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HENDERSON M A and PETTIGREW R T Induction of controlled hyperthermia in treatment of cancer Lancet (1971) 1275

HILL L Action of ultrashort waves on tumours Brit med J 2 (1934), 370

JARES J J and WARREN S L Cancer Problem Symposium p 225 1937

KLUFT O and BOEREMA I Hyperbaric oxygen in experimental cancer in mice Clinical appl cation of hyperbaric oxygen Proc 1st International Congress, Amsterdam, p 126 1963

KORB H Vergleichende Temperaturmessungen am Menschen bei den Wellenlängen 6 m und 1 m Strahlentherapie 64 (1939), 536

MANTILA L. MODES S and RENTCHNICK P In New trends in the treatment of cancer Springer Verlag Berlin 1967

MUCKLE D S The effect of hyperthermia on the growth and metabolism of the Vx2 carcinoma M S Th

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MÜLLER C. Die Röntgenstrahlenbehandlung der Malignen Tumoren und ihre Kombinationen Strahlentherapie III (1913), 177

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REFERENCES

ALLAIRE F J THIEME E T and KORST D R Cancer chemotherapy with 5-fluorouracil alone and in combination with X ray therapy Cancer Chemother Abstr 14 (1961) 59

ARONS I and SOKOLOFF B Combined roentgentherapy and ultrasound Amer J Surg 36 (1937) 533

BARTH G Radiology Book Abstract p 172 1956

BIRKNER R und WACHSMANN F Über die Kombination von Röntgenstrahlen und Kurzwellen Strahlentherapie 76 (1949), 93

CRILE G Selective destruction of cancers after exposure to heat Ann Surg 156 (1962), 404

— Criticism of conventional methods of treating solid tumours in man Brit med J 4 (1970), 489

FARBER S D'ANGIO G EVANS A and M[illegible] special reference to Wilms tumour in ch[illegible]

FOYE L V WILLET F M HALL B and Ro[illegible] 5 fluorouracil Cancer Chemother Abstr [illegible]

FUCHS H T Maligne Zelle und Embryonalzelle Klin Wschr 14 (1935) 1295

HENDERSON M A and PETTIGREW R T Induction of controlled hyperthermia in treatment of cancer Lancet (1971) 1275

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MUCKLE D S The effect of hyperthermia on the growth and metabolism of the Vx2 carcinoma M S Thesis University of Newcastle 1972

— and DICKSON J A The selective inhibitory effect of hyperthermia on the metabolism and growth of malignant cells Britt J Cancer XXV (1971) 771

MULLER C Die Rontgenstrahlenbehandlung der Malignen Tumoren und ihre Kombinationen Strahlentherapie III (1913) 177

MORRISON J M and JOHNSTON I D A Effect of combined I A Methotrexate and irradiation on the growth rate of the Vx2 carcinoma in rabbits Brit J Surg 55 (1968) 395

ROHDENBURG G L and PRIME F Effect of *combination radiation and heat on neoplasms* Arch Surg 2 (1921), 116

STEEL G G and LAMERTON L F Cell population kinetics and chemotherapy *In* Human tumour cell kinetics Nat Cancer Inst Monogr 30 (1969), 29

WARREN S L Preliminary studies of the effect of artificial fever upon hopeless tumour cases. Amer J Roentgenol 33 (1935), 75

WILDERMUTH O Hyperbaric radiation therapy in cancer management Radiology 82 (1964), 767

WOEBER K Combination X-ray and ultrasound treatment for dermatological conditions biological foundations and clinical application Amer J Phys Med 34 (1955), 376

FROM THE GENERAL DEPARTMENT, RADIUMHEMMET (DIRECTOR PROF JERZY EINHORN), KAROLINSKA SJUKHUSET, S-104 01 STOCKHOLM, SWEDEN

EFFECTS OF RADIATION THERAPY ON BLOOD-BORNE LEUCOCYTES IN PATIENTS WITH MAMMARY CARCINOMA

GORAN LUNDELL

Ionizing radiation is well known to cause changes in the bone marrow and peripheral blood, among other things in leucopenia (LAWRENCE et coll 1948, JACOBSON et coll 1949, BOND et coll 1965, LEHAR et coll 1966) The leucopenia is mainly attributed to a reduction in blood borne lymphocytes and segmented neutrophils ELVES (1970) has shown that antecedents to macrophages in the blood are highly resistant to irradiation in the rat, and it has earlier been revealed REBUCK et coll 1961, VOLKMAN & GOWANS 1965, VOLKMAN 1966) that the blood borne precursor to the macrophages probably takes the form of a small mononuclear cell or monocyte LEHAR et coll presented the results of local human bone marrow irradiation in nine patients, the number of blood borne monocytes remained virtually unchanged during the *irradiation* The same findings were reported by RENNER & HASSENSTEIN (1972)

The present report describes the changes in the amount of blood borne leucocytes in patients with mammary carcinoma after external radiation therapy

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Table 2

Number of leucocytes before radiation therapy in 48 patients

Cell type	Absolute number		Relative number	
	Mean value	SE	Mean, per cent	SE
Juvenile neutrophils	240	27	3.9	0.4
Segmented neutrophils	3160	177	51.2	1.4
Eosinophils	190	19	3.3	0.3
Basophils	50	5	0.8	0.1
Lymphocytes	2000	50	34.1	1.4
Monocytes	390	30	6.7	0.5
Total	6030	229	100.0	

clavicular regions were irradiated with ^{60}Co and the thoracic region with electrons having a mean energy of 12.5 MeV (10—15 MeV). The mammary regions received the prescribed dose in 5 weeks and the total irradiation time for both groups was 48 days (37—52). There was no significant difference as regards irradiation time between groups A and B. Using the data presented by MECHANIK (1926) it was estimated that approximately 10 per cent of the bone marrow was included in the treatment area. Thus, the major part of the radiation dose is given to the blood borne leucocytes.

The statistical calculations were performed using the Student's t test.

Results

No significant change in the number and relative differential of the leucocytes was observed in group B postoperatively as compared with preoperative values (Table 1). The results of tests before irradiation are given in Table 2. No significant differences were observed between the pre- and postoperative groups of patients regarding the number of leucocytes and their percentage differential before radiation therapy.

As expected, a significant reduction ($p < 0.001$) in the total number of blood borne leucocytes was observed after irradiation. This was due to the reduction in lymphocytes ($p < 0.001$) and segmented neutrophils ($p < 0.001$, Table 3). The number of juvenile neutrophils, eosinophils, basophils and monocytes for the combined group of patients remained virtually constant after radiation therapy. No significant difference between patients with right- and left sided carcinomas, respectively, is evident in Tables 1, 2 and 3.

A comparison was also made of the difference in the relative differential of

Table 1

Leucocyte changes after operation in 12 patients. Differences not significant

Cell type	No. of cases			Mean of individual leucocyte differences	
	Increase	Decrease	Unchanged	Mean, per cent	SE
Juvenile neutrophils	4	7	1	−0.6	0.6
Segmented neutrophils	5	6	1	−1.6	1.9
Eosinophils	7	5	0	+0.7	0.5
Basophils	4	4	4	0	0.2
Lymphocytes	6	6	0	+0.9	1.8
Monocytes	7	5	0	+0.6	1.0
Total, absolute values	8	4	0	+180	510

Material and Methods

The material consisted of 48 female patients from Radiumhemmet's series of mammary carcinoma, randomly selected so that 33 received preoperative (group A) and 15 postoperative irradiation (group B).

White blood cells were counted under standardized conditions 0 to 21 days (mean 14.8) before and 10 to 21 days (mean 14.0) after end of treatment. For group A, the postirradiation values were obtained before any surgical intervention had been undertaken, and for group B the time interval between the operation and the preirradiation leucocyte counts was at least three weeks. In 12 of the 15 patients in group B, the leucocytes were also determined immediately before and 21 to 49 days (mean 26.0) after the operation, but before radiation therapy.

The leucocytes were stained with May-Grunwald-Giemsa and the total number of leucocytes and their percentage differential were determined. For practical reasons, there was no possibility to have all the tests counted in the morning with the patients fasting. In a single case, the blood tests before and after the radiation therapy were performed at the same time of the day in order to minimize the changes in the leucocyte count during the day. The patients had a mean age of 55 years, the youngest being 36 and the oldest 70 years of age, 25 had a left-sided and 23 a right-sided carcinoma.

The dose given was 4 500 rad to the mammary, axillary and supraclavicular regions with a tumour dose of 175 rad at each irradiation. For the preoperative group, ^{60}Co was used throughout and the mammary gland was irradiated using two opposed wedge-fields, for the postoperative group the axillary and supra-

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A comparison was also made of the difference in the relative differential of

Table 3

Leucocyte changes after radiation therapy in 48 patients Absolute numbers

Cell type	No of cases			Mean of individual leucocyte differences		Significance of the difference
	Increase	Decrease	Unchanged	Mean	SE	p
Juvenile neutrophils	27	21	0	+19	33	–
Segmented neutrophils	12	36	0	−733	175	< 0.001
Eosinophils	22	25	1	+1	25	–
Basophils	23	24	1	+7	10	–
Lymphocytes	1	47	0	−1127	87	< 0.001
Monocytes	*26*	*22*	*0*	*+21*	*36*	–
Total	4	44	0	−1812	266	< 0.001

the leucocyte values before and after irradiation For the lymphocytes, a highly significant reduction was observed ($p < 0.001$) A highly significant increase ($p < 0.001$) of the juvenile and segmented neutrophil and monocyte cells occurred Eosinophil and basophil values were significantly increased ($p < 0.01$ and $p < 0.05$, respectively, Table 4)

Discussion

In the peripheral blood monocytes are considered to be the most difficult cells to differentiate from other leucocytes and especially from neutrophils and me-

Table 4

Leucocyte changes in per cent after radiation therapy in 48 patients

Cell type	No of cases			Mean of individual leucocyte differences		Significance of the difference
	Increase	Decrease	Unchanged	Mean	SE	p
Juvenile neutrophils	32	10	6	+2.3	0.5	0.001
Segmented neutrophils	35	13	0	+5.5	1.4	0.001
Eosinophils	28	12	8	+1.5	0.5	< *0.01*
Basophils	25	13	10	+0.4	0.2	< 0.05
Lymphocytes	4	44	0	−13.0	1.4	< 0.001
Monocytes	37	10	1	+3.3	0.6	< 0.001

dium sized or large lymphocytes (DIGGS et coll 1954, COPENHAVER & JOHNSON 1958) A percentage increase in juvenile and segmented neutrophils and monocytes might then be of importance in immunologic investigations as the results are often given as stimulation values for a certain number of lymphocytes Thus, a false value might be obtained if the lymphocytes are not properly isolated from the monocytes, as may occur for instance when lymphocyte stimulation values before and after radiation therapy are compared

Several authors have reported an increase in the number of peripheral eosinophils after irradiation, especially when treating the abdominal region (MORCZEK & CHRISTOPH 1958, CHONE & FUCHS 1969) On the other hand, unchanged or decreased eosinophil counts have also been reported (SPIETHOFF 1930, PARLAVECCHIO 1940, RENNER & HASSENSTEIN) In the present material no significant differences were observed after irradiation in the number of eosinophils, basophils and monocytes, nor any significant change in the number and relative distribution of the leucocytes in group B postoperatively as compared with the preoperative values after irradiation As no difference was found between right and left sided carcinomas, it is hardly conceivable that the position of the thoracic duct would influence the results obtained

Acknowledgements

The author wishes to thank Prof J Einhorn for his valuable advice and Dr Ulla Glas for her cooperation in obtaining the leucocyte counts

SUMMARY

The results of absolute and relative leucocyte counts in 48 pre or postoperatively irradiated patients with mammary carcinoma have been evaluated A reduction in the number of lymphocytes and segmented neutrophils occurred whereas the juvenile neutrophils eosinophils, basophils and monocytes remained unchanged In the differential leucocyte counts a significant increase of juvenile and segmented neutrophils monocytes eosinophils and basophils was encountered however These results may be of importance in immunologic investigations when lymphocyte stimulation values before and after irradiation are evaluated

ZUSAMMENFASSUNG

Die Ergebnisse der absoluten und relativen Leukozytenwerte bei 48 prä oder postoperativ bestrahlten Patienten mit einem Brustkarzinom sind zusammengestellt worden Es trat eine Verringerung der Zahl der Lymphozyten und segmentformigen Neutrophilen auf während die jungen Neutrophilen Eosinophilen Basophilen und Monozyten unverändert blieben Bei den Differential Leukozytenwerten stiegen die jungen und segmentformigen Neutrophilen Monozyten Eosinophilen und Basophilen jedoch Diese Ergebnisse mogen von Bedeutung in immunologischen Untersuchungen sein wenn Lymphozyten Stimulierungswerte vor und nach Bestrahlung festgestellt werden

Table 3

Leucocyte changes after radiation therapy in 48 patients. Absolute numbers

Cell type	No. of cases			Mean of individual leucocyte differences		Significance of the difference
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the leucocyte values before and after irradiation. For the lymphocytes, a highly significant reduction was observed ($p < 0.001$). A highly significant increase ($p < 0.001$) of the juvenile and segmented neutrophil and monocyte cells occurred. Eosinophil and basophil values were significantly increased ($p < 0.01$ and $p < 0.05$, respectively, Table 4).

Discussion

In the peripheral blood monocytes are considered to be the most difficult cells to differentiate from other leucocytes and especially from neutrophils and me-

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Leucocyte changes in per cent after radiation therapy in 48 patients

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FROM THE DEPARTMENT OF IMMUNOLOGY, THE GENERAL INFIRMARY, UNIVERSITY OF LEEDS, THE REGIONAL RADIOTHERAPY CENTRE, COOKRIDGE HOSPITAL, AND THE DEPARTMENT OF HAEMATOLOGY, UNITED LEEDS HOSPITALS, LEEDS LS1 3EX ENGLAND

PROGRESSIVE LOSS OF IMMUNE STATUS AND EMERGENCE OF AUTO-ANTIBODIES FOLLOWING RADIATION THERAPY ON HODGKIN'S DISEASE

G GOWLAND, J STONE, B E ROBERTS, JACQUELINE WILSON and LESLEY COOK

In certain stages of Hodgkin's disease radiation therapy is the treatment of choice The patient, with stage IIb reported below had a series of dose-response lymphocyte transformation tests and other immunologic investigations performed before and at various times after irradiation until she finally succumbed to her disease The influence of irradiation on both the immune status of the individual and the appearance and significance of thyroid antibodies are discussed

Case Report

A 72 year-old female noticed a swelling of the neck which gradually increased in size over the subsequent two months before she was first seen as an out patient on 8 Aug 1972 She had lost about 7 kg in weight in 8 months and had had freq[illegible] tempera ture [illegible]

On [illegible]

was [illegible] is pressure

[illegible] She was

Submitted for publication 1 April 1974

RÉSUMÉ

L'auteur examine les résultats de numérations leucocytaires absolue et relative chez 48 malades atteintes de cancer du sein et irradiées avant ou après l'opération. Il a constaté une réduction du nombre des lymphocytes et des neutrophiles segmentés alors que les neutrophiles jeunes, les éosinophiles, les basophiles et monocytes restent sans changement. Il a constaté cependant dans la numération leucocytaire différentielle une augmentation *significative des neutrophiles jeunes et segmentés* des monocytes des éosinophiles et des basophiles. Ces résultats peuvent être d'importance dans des études immunologiques pour évaluer les valeurs de stimulation lymphocytaire avant et après irradiation.

REFERENCES

BOND V P, FLIEDNER T M and ARCHAMBEAU J O Mammalian radiation lethality a disturbance in cellular kinetics Academic Press New York and London 1965

CHONÉ B und FUCHS H B Verlaufsuntersuchungen am Leukozytenkonzentrat während *und nach therapeutischer Strahleneinwirkung Berlin Radiobiol Radiother 10 (1969)* 489

COPENHAVER W M and JOHNSON D D Blood lymph Bailey's textbook of histology Williams & Wilkins Co Baltimore 1958

DIGGS L W, STURM D and BELL A The morphology of blood cells What's New (1954) 183

EVES M W Some observations on the life history of the macrophage J Path 101 (1970) 333

JACOBSON L O, MARKS E K and LORENZ E The hematological effects of ionizing radiations Radiology 52 (1949) 371

LAWRENCE J S, DOWDY A H and VALENTINE W N Effects of radiation on hemopoiesis Radiology 51 (1948) 400

LEHAR T J, KIELY J M, PEASE G L and SCANLON P W Effect of focal irradiation on human bone marrow Amer J Roentgenol 96 (1966) 183

MECHANIK N Untersuchungen über das Gewicht des Knochenmarkes des Menschen Z ges Anat 79 (1926) 58

MORCZEK A und CHRISTOPH I Verhalten der eosinophilen Leukozyten im peripheren Blutbild unter der Einwirkung einer hochdosierten Röntgenbestrahlung Strahlentherapie 105 (1958) 419

PARLAVECCHIO A Considerazioni ed osservazioni sui metodi di cura delle leucemie croniche *e sulle modificazioni ematologiche indotte dalla radioterapia nelle mielosi leucemiche* Policlinico Sez med 47 (1940) 194

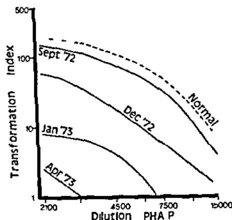
REBUCK J W, PETTS A J, RIDDEL J M, PRIEST R H and LOGRIPPO J H Human leucocyte functions in the tissues *In* Biological activity of the leucocyte Ciba Foundation Study Group No 10 (1961)

RENNER H und HASSENSTEIN E Blutbildveränderungen während der postoperativen Lymphabflussbestrahlung bei Seminompatienten Strahlentherapie 144 (1972) 595

SPIETHOFF B *Die Allgemein Behandlung mit Grenzstrahlen nach Bucky und der Einfluss der* Grenzstrahlen auf das Blutbild Münch med Wschr 77 (1930) 219

VOLKMAN A The origin and turnover of mononuclear cells in peripheral exudates in rats J exp Med 124 (1966) 241

— and GOWANS J The production of macrophages in the rat Br J Exp Path 46 (1965) 50



Dose response curves to seven dilutions of PHA P performed at various times Transformation Index — av counts experimental — av counts unstimulated controls/av counts unstimulated controls

Hodgkin s disease) were observed She recovered only to be readmitted about one month later with bowel obstruction and an enlarged liver and spleen Management was conservative and she died after three days (on 13 June 1973)

Immunologic observations Haematologically the patient apparently recovered well from the irradiation and by 5 Dec 1972 (4 weeks after end of treatment) her blood picture was normal By 30 Jan 1973 (8 weeks later) she had developed a lymphopenia, but then remained relatively stable over the next three months A marked depression in the lymphocyte count occurred shortly before her death (Table 1)

Lymphocyte transformation tests were performed on the equivalent of 0 1 ml aliquots of the patient's whole blood in 3 ml volumes of Medium 199 supplemented with either 20 per cent pooled human AB or patient's own serum Seven dilutions of PHA P (Difco batch No 575219) were used in each test with quadruplicate cultures for each dilution, 7 unstimulated controls were included Transformation was assessed using ^{3}H-thymidine (Amersham), labelling at 72 h, processing and counting were performed as described by HUGHES & CASPARY (1970) The results of these tests together with a curve for a normal control female are presented in the Figure

Before irradiation the patient s dose response curve was sub normal but not seriously so However after therapy, and at a time when her blood picture had apparently returned to normal, the ability of her lymphocytes to transform with PHA was found to be seriously impaired (about 25 per cent of her pre irradia-

Table I

Cell counts × 10^9 per ml

	Total	Neutro-phils	Mono-cytes	Lympho-cytes	Platelets
19 Sept 1972	7 1	5 2	0 36	1 3	281
Irradiation 13 Oct 1972 to 9 Nov 1972					
16 Nov 1972	3 6	2 7	0 18	0 6	160
5 Dec 1972	7 1	5 0	0 43	1 6	283
30 Jan 1973	7 4	5 9	0 59	0 52	264
24 April 1973	15 8	14 1	0 79	0 63	454
23 May 1973	8 0	7 3	0 40	0 08	315

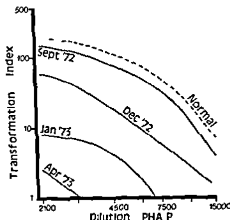
not in cardiac failure Multiple nodes (1 to 3 cm diameter) were palpable in the neck and the thyroid gland was enlarged especially on the right side where it measured 3 cm × 2 cm There were a few 1 cm nodes in the axillae and the groins were clear

Chest films demonstrated smooth enlargement of the superior mediastinum, skeletal survey, liver and spleen scan were all normal Thyroid scan showed no activity on the right, but a little activity on the left At urography and angiography the right kidney was found to be larger than the left due to a probable benign cyst and bilateral small artery disease Creatine clearance was 33 ml per min, all other biochemical tests were within the normal range for her age, except serum cholesterol (364 mg/100 ml) Her blood picture was also normal except for a slightly raised ESR (22 mm in the first hour)

Following excision biopsy of a node on the left side of the neck, the patient was diagnosed as having stage IIb Hodgkin's disease of mixed type (Lukes classification) with superior vena cava obstruction

Treatment A radical course of irradiation on the 4 MeV linear accelerator using the mantle technique (treating nodes in the neck, axillae, mediastinum and upper abdomen) was given between 13 Oct 1972 and 9 Nov 1972 A median tumour dose of 4 000 rad was given in 28 days with a maximum spinal cord dose of 3 700 rad and full lung and kidney protection

Clinical progress There was a good response to the irradiation with complete disappearance of all nodes and the thyroid swelling However, the general condition of the patient deteriorated a little, she lacked energy, was anorexic and lost a little more weight Thereafter she improved steadily and appeared very well clinically on 30 Jan 1973 (11 weeks after treatment) Three months later (on 6 May 1973) she was admitted with perforation of the small intestine which was operated upon The walls of the gut appeared to be infiltrated by Hodgkin's disease and enlarged mesenteric nodes (proved histologically to contain active



Dose response curves to seven dilutions of PHA P performed at various times Transformation Index = av counts experimental — av counts unstimulated controls/av counts unstimulated controls

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Table 2

Immunoglobulin levels and auto antibodies before and at various times after mantle treatment

	Immunoglobulins mg/100 ml				Auto antibodies					
	G	A	M	E*	Thyroid microsomal		Thyroglobulin	'Rheumatoid factor'		
					IF	CFT	TCAT	Latex 1	Latex 2	SCAT
19 Sept 1972	1 200	120	23	310			1 10		–	–
	Irradiation 13 Oct 1972 to 9 Nov 1972									
16 Nov 1972	Not done				+		1 2 500	++++		–
5 Dec 1972	1 200	130	20	270	+		1 5 000	++++		
30 Jan 1973	700	70	30	110	+		1 10 000	+++		
21 April 1973	600	65	9	< 100	+		> 1 10 000	+++		

* IU/ml

I F immunofluorescent test C F T —complement fixation test, T C A T —tanned cell agglutination test

S C A T sheep cell agglutination test for rheumatoid factor

Latex 1 latex particles sensitised with patient's own gamma globulin

Latex 2—latex particles sensitised with pooled human gamma globulin

tion response (5 per cent of the level by 30 Jan 1973, 1 4 per cent by 21 April 1973) which could not be accounted for by her apparently stable lymphopenia at this time, but in retrospect was probably coincident with disseminated Hodgkin's disease At no time did the patient's own serum exhibit a lymphocyte transformation inhibitor

Before radiation therapy the patient's serology was reasonably normal for a woman of her age, a low IgM and a slightly subnormal IgA being the only things of note From 5 Dec 1972 onwards however her immunoglobulins progressively but slowly decreased in concentration (Table 2) By the end of the treatment several auto-antibodies were detectable (1 an anti-thyroglobulin which increased in titre throughout the observation time, (2) an antibody to thyroid microsomes consistently detectable by immunofluorescence but not by complement fixation and (3) a peculiar 'rheumatoid factor' reactive with her own denatured gamma-globulin but not with denatured pooled human gamma-globulin or native rabbit gamma-globulin This latter is a finding for which

there is as yet no explanation, but is a consistent finding in the Hodgkin's patients which we have examined

Discussion

It would appear that this is a case in which the immune status of the patient with respect to cell mediated immunity (T-lymphocyte response as detected by the transformation test) was markedly and permanently affected as a consequence of irradiation and advanced Hodgkin's disease, and where apparently lymphocyte transformation was the only really accurate guide to the patient's true clinical condition

The following might be a reasonable explanation of the observed results Irradiation resulted in thyroid damage leading to the leakage of tissue specific antigens and also to the death of large numbers of both T- and B lymphocytes and injury to many more cells which then gradually died off This would initially show as a reduced transformation response due to both a decrease in numbers of T cells and malfunction in the injured survivors, followed by a progressive decrease in response as the damaged T-cells died off (Figure) Due to lack of thymus function on account of both age and irradiation damage, the patient was unable to replace these lost thymus-dependent lymphocytes Under these circumstances the patient would be unable to synthesize immunoglobulins efficiently, combat the spread of her Hodgkin's disease and would be defenceless against infective agents which would normally evoke either cell-mediated immunity or immunity dependent on T cell—B-cell co-operation

On the other hand, replacement of B cells would take place (until the system was impaired by progressing Hodgkin's disease, see COHNEN et coll 1973) There would thus then be the possibility of both old and newly generated B-cell clones in the patient s immune system potentially capable of reacting against thyroid antigens Thyroid antibodies might then ensue due to the removal (and non-replacement) of a population of T cells previously tolerant of thyroglobulin, and the direct stimulation of reactive B cells by the raised level of circulating thyroid antigen following irradiation damage (ALLISON et coll 1971, WEIGLE 1971, URBANIAK et coll 1973)

In using irradiation to treat this patient adequate dosage was important to prevent recurrence of symptoms and in particular superior vena cava obstruction However, as demonstrated here, the tolerance of elderly patients to irradiation is reduced and this may result in far reaching effects on the immune system This case illustrates that the routine estimation of lymphocyte performance may in some instances be the best way of monitoring clinical progress and hence be an accurate guide to patient management

Acknowledgements

This case is part of an on going project supported by a grant from the Board of Governors of the United Leeds Hospitals The patient was referred to us by Dr Tait Dewsbury General Hospital, Dewsbury

SUMMARY

A case of stage IIb Hodgkin s disease in a 72 year old female patient who was irradiated is described It is postulated that *radiation therapy induced a progressive depression* in her immune status with respect to cell mediated (T cell) response This in turn resulted in a loss of low dose tolerance of auto antigens and the production of auto antibodies Attention is drawn to the possible consequences of irradiation particularly in the elderly and the potential value of serial monitoring of immune status as a guide to clinical progress and management

ZUSAMMENFASSUNG

Der Fall einer Grad IIb Hodgkin scher Erkrankung bei einer 72 jährigen Patientin die bestrahlt worden war wird beschrieben Es wird festgestellt, dass die Strahlentherapie eine *fortschreitende Herabsetzung des Immunstatus hinsichtlich der Zell gebundenen (T Zellen)* Reaktivität hervorgerufen hat Diese führte ihrerseits zu einem Verlust der niedrigen Dosis Toleranz der Autoantigene und der Produktion von Autoantikorpern Es wird auf die möglichen Konsequenzen der Bestrahlung besonders bei älteren Patienten hingewiesen und auf den potentiellen Wert der wiederholten Feststellung des Immunstatus als Richtlinie *des klinischen Verlaufs und der Behandlung*

RÉSUMÉ

Présentation d un cas de stade II b de maladie de Hodgkin chez une femme âgée de 72 ans traitée par irradiation Les auteurs admettent que l irradiation a amené une dépression progressive de son état immunitaire en ce qui concerne la réponse *par l intermédiaire de* cellules (cellules T) Cette dépression a eu pour conséquence une diminution de la tolérance aux faibles doses d auto antigènes et la production d auto anticorps Les auteurs attirent l attention sur les conséquences possibles de l irradiation en particulier chez les sujets âgés et sur la valeur possible de contrôles en série de l état immunitaire comme guide de l évolution *clinique et du traitement*

REFERENCES

ALLISON A C DENMAN A M and BARNES R D Co operating and controlling functions of thymus derived lymphocytes in relation to autoimmunity Lancet 1971 II p 135

COHNEN G KONIG E AUCENER W and BRITTINGER G Lymphocyte response to pokeweed mitogen in Hodgkin s disease Biomedicine (Exp) 19 (1973) 239

HUGHES D and CASPARY E A Lymphocyte transformation in vitro measured by tritiated thymidine uptake I Lymphocyte culture techniques Int Arch Allergy 37 (1970) 506

URBANIAK S J PENHALE W J and IRVINE W J Circulating lymphocyte subpopulations in Hashimoto thyroiditis Clin Exp Immunol 15 (1973) 345

WEIGLE W O Recent observations and concepts in immunological unresponsiveness and auto immunity Clin exp Immunol 9 (1971) 437

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DARK RETICULAR CELLS IN THE THYMUS OF MICE

BERTIL JARPLID

The increased knowledge of the functional importance of the mammalian thymus has caused an increased interest in the morphology of this organ in general and in its ultrastructure in particular. Thus several reports on this subject have been published in the last decade (CLARK 1963, WEISS 1963, LUNDIN & SCHELIN 1965, VAN HAELST 1967, MANDEL 1968, ABE & ITO 1970, PEREIRA & CLERMONT 1971, RAPPAY et coll. 1971). The investigations have been concentrated to the most common types of cells, the lymphocytes, the epithelial reticular cells and the mesenchymal reticular cells or macrophages. Little interest has been devoted to a less common, dark reticular cell with increased electron density (IZARD & DE HARVEN 1968). The identity of this cell is not completely known, nor is its function or its relationship to other thymic cells. This short report will elucidate some details of the morphology of this dark cell and of its distribution in normal and in irradiated thymus and in thymic lymphoma of mice.

Material and Methods. C_3H and CBA mice, aged 20 days to 4 months were used. For radiation experiment a total dose of 550 R was divided between four equal exposures and given every fifth day beginning at an age of 25 days. Lymphomas were induced by fractionated irradiation or by a combination of fractionated irradiation and injection of ^{90}Sr (JARPLID 1974). The irradiation

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Fig. 1. Plenty of dark cells in the medulla (above) and in the cortex near the boundary between cortex and medulla. Toluidine blue stain. × 165



Fig. 2. Uneven distribution of dark cells in the cortex (below) and medulla. A capillary right. Toluidine blue stain. × 165

conditions were described previously (JARPLID 1968). Specimens from the thymus were fixed in Stieve's fluid and stained with Ehrlich's haematoxylin and eosin for light microscopy. For electron microscopy specimens were fixed in glutaraldehyde or formaldehyde-glutaraldehyde solution (KARNOVSKY 1965), postfixed in OsO_4 (BENNETT & LUFT 1959) and embedded in Epon (LUFT 1961). For light microscopy 1 μ sections were stained with toluidine blue (BJÖRKMAN 1962). Ultrathin sections were stained with uranylacetate in methanol (STEMPAK & WARD 1964).



Fig 3 Thymic cortex Dark cells only along a connective tissue structure left Toluidine blue stain ×470

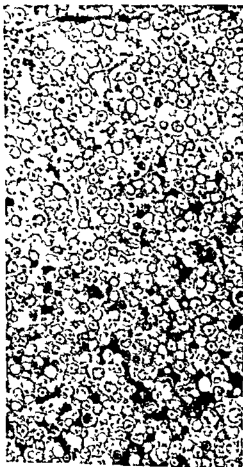


Fig 4 Thymic lymphoma Uneven distribution of dark cells Toluidine blue stain ×470

and lead citrate (VENABLE & COGGESHALL 1965) and examined in a Siemens Elmiskop IA at 60 kV

Results

In light microscopy the most evident characteristics of the dark cells were the affinity to toluidine blue stain The cells were irregularly stellate and sometimes had long slender processes Both the nucleus and the cytoplasm were very dark and the nuclear membrane was often indistinct (Figs 1—4)



Fig 5

Fig 6

Fig 5 Light epithelial reticular cell with cytoplasmic processes (P), intracytoplasmic fibrils (F), a desmosome (inserted ×23 250) and vacuoles (V) ×6 720

Fig 6 Dark epithelial reticular cell characterized by cytoplasmic processes and increased density of both nucleus and cytoplasm Vacuoles (V) fibrillar structures (inserted (F), ×23 040) and plenty of ribosomes in the cytoplasm Desmosome (inserted ×23 040) ×9 600



Fig 7 Dark reticular cell with slender cytoplasmic processes The nucleus is homogenous and the density is equalized between nucleus and cytoplasm Possible cellular degeneration × 16 100

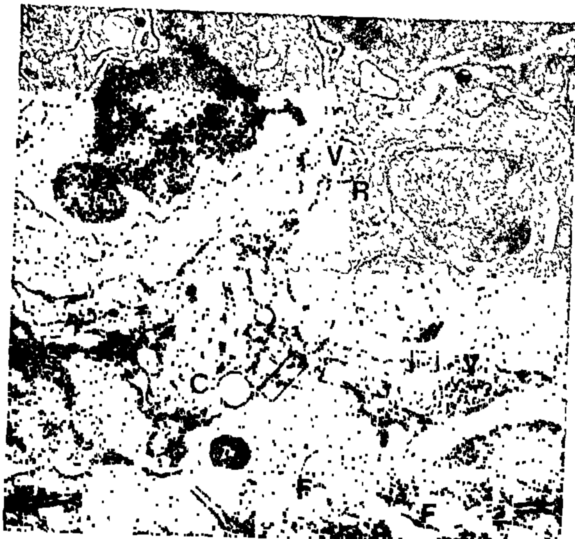


Fig. 8. Cytoplasmic processes of dark epithelial reticular cell in contact with connective tissue structures (C) and containing vacuoles (V), accumulations of ribosomes (R), fibrillar structures (inserted, F, ×36 720) and several desmosomes (inserted ×36 720). ×12 240.

Also in electron microscopy the cells were dark as a result of increased electron density compared to surrounding cells, both in the nucleus and in the cytoplasm (Figs. 5—7). The nucleus was polygonal, ovoid or somewhat elongated. In some cases the nucleus was homogeneous with a density similar to that of the cytoplasm and with an indistinct nuclear membrane (Fig. 7). The density of the cytoplasm could be referred to the uniformly granular cytoplasmic matrix (Fig. 9). The number of organelles varied. Plenty of ribosomes and some mitochondria were generally seen. Many cells had vacuoles, sometimes containing an amorphous material of moderate density (Figs. 6, 8). The slender processes extending between



h desmosomes fibrillar struc
finely granular hyaloplasm
)0) and c) (×59 200) are

surrounding cells had a finely granular cytoplasmic matrix with plenty of ribosomes, often unevenly distributed, and sometimes fibrillar structures (Figs 8, 9) Desmosomes were sometimes observed in connection with adjacent epithelial cells or within the dense matrix (Figs 6, 8, 9)

The frequency and the distribution of the dark cells varied greatly both between different animals and between different sections from the same thymus (Figs 1, 2) In some sections no dark cells were observed In other sections they generally appeared as single cells, in the cortex often related to light epithelial cells, to capillaries or to connective tissue structures (Figs 2, 3, 8) Generally they were more common near the boundary between the cortex and the medulla than near the capsule Sometimes the cells appeared in accumulations or in lines along interlobular septa (PEREIRA & CLERMONT 1971) in the cortex (Fig 3) In the medulla the dark cells generally appeared as single cells often in contact with epithelial cells

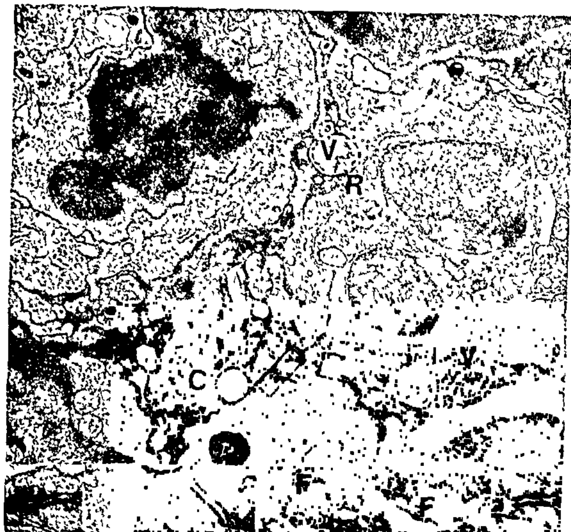


Fig 8 Cytoplasmic processes of dark epithelial reticular cell in contact with connective tissue structures (C) and containing vacuoles (V), accumulations of ribosomes (R), fibrillar structures (inserted, F, × 36 720) and several desmosomes (inserted, × 36 720) × 12 210

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MONT 1971) The frequent observation of dark cells related to capillaries and connective tissue structures (Figs 2, 8), which have also been reported previously (IZARD & DE HARVEN, MOLLO et coll), and the observation of many intermediate forms between dark and light epithelial reticular cells (IZARD & DE HARVEN) mentioned above, indicate an intimate relationship between these cells

The homogenisation of the nuclear structures and the equalized density between nucleus and cytoplasm in some dark cells may indicate degeneration No similar or other appearances indicating degeneration were observed in light epithelial cells This may suggest that dark cells are degenerating epithelial reticular cells (MOLLO et coll)

Dark cells did not increase during regeneration of the radiation injured thymus, nor did they seem to be more common in thymic lymphomas than in normal thymus In contrast to this IZARD & DE HARVEN found an increased number of dark cells in thymic lymphomas of AKR mice

In conclusion the hypothesis may be suggested that dark and light epithelial reticular cells may be different modes of expression of the same cell type

Acknowledgement

This investigation was carried out as part of the programme of the European Late Effects Project Group (EULEP)

SUMMARY

The morphology and distribution of dark reticular cells in the thymus of normal *mice of*

ZUSAMMENFASSUNG

Die Morphologie und Verteilung der dunklen retikularen Zellen im Thymus von normalen und bestrahlten Mausen und von Mausen mit einem Lymphom des Thymus werden beschrieben Der Verfasser kam zum Schlusssatz, dass die dunklen Zellen epitheliale retikulare Zellen sind und legte die Hypothese vor, dass die dunklen und hellen epithelialen retikularen Zellen verschiedene Formen des selben Zelltypus darstellen

RÉSUMÉ

Description de la morphologie et de la distribution des cellules reticulaires somb
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After irradiation the frequency of the dark cells did not seem to increase during the different phases of depletion and regeneration of the thymus (JARPLID 1968), whether the frequency decreased was difficult to establish because of the normally irregular and low frequency of these cells

In the thymic lymphomas the dark cells had the same appearances as they did in normal thymus (Fig 4) Three out of six cases of localized thymic lymphoma had a few dark cells and one had plenty of them Among 10 cases of generalized thymic lymphoma one had numerous dark cells and another case only a few Eight of these tumours had no dark cells

Discussion

Cells similar to these dark cells have previously been described as 'dense reticular cells' in thymus and lymph nodes of mice (IZARD & DE HARVEN 1968) and as 'dark reticular cells' in lymphadenitis and lymphomas of man (MOLLO et coll 1969) HIROKAWA (1969) found two types of epithelial reticular cells in the thymus of human foetus and newborns, one 'hypertrophic' type which was seen only in the medulla and one 'slender' type This slender type had long, cytoplasmic projections and a nucleus which was large and pale when the cell appeared in the cortex but smaller and darker when the cell appeared in the medulla In the present material the dark cells were of similar appearance in the cortex and in the medulla RAPPAY et coll (1971) found it difficult to analyse the fine structure of slender cells in the thymus of perinatal rats because of the great density of these cells

The dark cells have several characteristic features suggestive of light epithelial reticular cells, viz long, slender cytoplasmic processes, desmosomes, vacuoles and fibrillar structures in the cytoplasm, and a similar distribution in cortex and medulla A close relationship between these two cells was also suggested by the observation of many intermediate forms of variable size and density in the same sections (IZARD & DE HARVEN) These authors did not notice any occurrence of desmosomes, however, and the densities found by MOLLO et coll between different contacting dark processes could not with certainty be interpreted as desmosomes In the present material, however, the desmosomes both within the dense cytoplasmic matrix itself and between dark cells and light epithelial cells are evident and indicate that these dark cells are true epithelial reticular cells Similar dark cells in the thymus of chicken have also been interpreted as epithelial cells (FRAZIER 1973)

The epithelial reticular cells constitute the supporting framework of the cortex and outer medulla and separate these regions from the connective tissue of the capsule, interlobular septa, blood vessels and inner medulla (PEREIRA & CLER

MONT 1971) The frequent observation of dark cells related to capillaries and connective tissue structures (Figs 2, 8), which have also been reported previously (IZARD & DE HARVEN, MOLLO et coll), and the observation of many intermediate forms between dark and light epithelial reticular cells (IZARD & DE HARVEN) mentioned above, indicate an intimate relationship between these cells

The homogenisation of the nuclear structures and the equalized density between nucleus and cytoplasm in some dark cells may indicate degeneration No similar or other appearances indicating degeneration were observed in light epitheliar cells This may suggest that dark cells are degenerating epithelial reticular cells (MOLLO et coll)

Dark cells did not increase during regeneration of the radiation injured thymus, nor did they seem to be more common in thymic lymphomas than in normal thymus In contrast to this IZARD & DE HARVEN found an increased number of dark cells in thymic lymphomas of AKR mice

In conclusion the hypothesis may be suggested that dark and light epithelial reticular cells may be different modes of expression of the same cell type

Acknowledgement

This investigation was carried out as part of the programme of the European Late Effects Project Group (EULEP)

SUMMARY

The morphology and distribution of dark reticular cells in the thymus of normal mice of irradiated mice and of mice with thymic lymphoma are described It is concluded that dark cells are epithelial reticular cells and the hypothesis is suggested that dark and light epithelial reticular cells may be different modes of expression of the same cell type

ZUSAMMENFASSUNG

Die Morphologie und Verteilung der dunklen retikularen Zellen im Thymus von normalen und bestrahlten Mausen und von Mausen mit einem Lymphom des Thymus werden beschrieben Der Verfasser kam zum Schlusssatz dass die dunklen Zellen epitheliale retikulare Zellen sind und legte die Hypothese vor dass die dunklen und hellen epithelialen retikularen Zellen verschiedene Formen des selben Zelltypus darstellen

RÉSUMÉ

Description de la morphologie et de la distribution des cellules reticulaires sombres dans le thymus de souris normales de souris irradiees et de souris atteintes de lymphome thymique L auteur conclut que les cellules sombres sont des cellules reticulaires epitheliales et il émet l hypothese que les cellules reticulaires epithéliales sombres et claires peuvent être des modes differents de l expression du même type de cellules

REFERENCES

ABE K and ITO T Fine structure of small lymphocytes in the thymus of the mouse Qualitative and quantitative analysis by electron microscopy *Z Zellforsch* 110 (1970) 321

BENNETT H S and LUFT J H S collidine as a basis for buffering fixatives J biophys biochem Cytol 6 (1959), 113

BJORKMAN N Low magnification electron microscopy in histological work Acta morph neerl scand 4 (1962) 344

CLARK S L The thymus in mice of strain 129/J studied with the electron microscope Amer J Anat *112* (1963), 1

FRAZIER J A Ultrastructure of the chick thymus *Z Zellforsch* 136 (1973) 191

VAN HAELST U J G M De normale thymus en zijn veranderingen tijdens de experimentele *snelle involutie en regeneratie Een lichten electronenmicroscopisch onderzoek bij de rat* (In Dutch) Thesis Nijmegen 1967

HIROKAWA K Electron microscopic observation of the human thymus of the fetus and the newborn Acta path jap 19 (1969) 1

IZARD J and DE HARVEN E Increased numbers of a characteristic type of reticular cell in the thymus and lymph nodes of leukemic mice An electron microscope study Cancer Res 28 (1968) 421

JARPLID B Radiation induced asymmetry and lymphoma of thymus in mice *Acta radiol* (1968) Suppl No 279

Combined effect of roentgen irradiation and radiostrontium on the haematopoietic tissues and the development of lymphoma in mice Acta radiol Ther Phys Biol 13 (1974) 217

KARNOVSKY M J A formaldehyde glutaraldehyde fixative of high osmolality for use in electron microscopy J cell Biol 27 (1965) 137

LUFT J H Improvements in epoxy resin embedding methods J biophys biochem Cytol 9 (1961) 409

LUNDIN P and SCHELIN U Ultrastructure of the rat thymus Acta path microbiol scand 65 (1965) 379

MANDEL T Ultrastructure of epithelial cells in the medulla of the guinea pig thymus Aust J exp Biol med Sci 46 (1968) 755

MOLLO F MONGA G and STRAMIGNONI A Dark reticular cells in human lymphadenitis and lymphomas Virchows Arch Abt B Zellpath 3 (1969) 117

PEREIRA G and CLERMONT Y Distribution of cell web containing epithelial reticular cells in the rat thymus Anat Rec 169 (1971) 613

RALLAY G BUKULYA B and BACSY E Fine structure distribution and function of the rat thymic reticular cells in the perinatal life Acta biol Acad Sci hung 22 (1971) 187

STEMPAK G and WARD T An improved staining method for electron microscopy J cell Biol *22* (1964) 697

VENABLE J H and COGGESHALL R A A simplified lead citrate stain for use in electron microscopy J cell Biol *25* (*1965*) *407*

WEISS L Electron microscopic observations on the vascular barrier in the cortex of the thymus of the mouse Anat Rec 145 (1963) 413

FROM THE DEPARTMENTS OF RADIATION THERAPY AND ANATOMY, UMEÅ UNIVERSITY, S-901 85 UMEÅ, SWEDEN

ELECTRON MICROSCOPY OF BONE MARROW FOLLOWING THE INTRAVENOUS INJECTION OF COLLOIDAL GOLD

N-E SÄTERBORG and EBBA CEDERGREN

Colloidal gold injected intravenously will be quickly removed from the circulation by the reticuloendothelial system, with colloidal gold stabilized with gelatine and of uniform small particle size (DEL TURCO & PIETRA 1960) 90 per cent will be accumulated in the liver and spleen and the remainder in the bone marrow (SHEPPARD et coll 1947) SÄTERBORG (1971) demonstrated by autoradiography that gold particles injected intravenously were concentrated in the sinusoidal lining cells of the bone marrow of rabbit An extrasinusoidal diffuse distribution of active colloidal gold particles will be registered in the film only as a high background deposition of silver grains and colloidal ^{198}Au will thus fail to disclose whether the colloid plays any part in an open or closed circulation of the bone marrow, this is of some clinical interest The incorporation of colloidal particles into the cells may occur by the uptake of extracellular material into membrane bound vesicles or by simple transfer of particles through the cell membranes (WISSING 1958, MOORE et coll 1961, KAYE & PAPPAS 1962) processes between [illegible]
may sometimes als[illegible]
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Submitted for publication 24 April 1973

Electron microscopy makes the identification of single gold particles as well as their situation in different cells and cell membranes possible. The present electron microscopy was undertaken to obtain detailed information about the distribution in the bone marrow of colloidal gold injected intravenously. Rabbits were used as the arrangement, the blood formation and composition of bone marrow in these animals and man being similar (VON HERRATH 1954, FRESEN & LIEBENFELD 1956, FLEICHER 1962).

Material and Method. Twelve rabbits weighing 2 500 to 3 000 g were used. Nine of these were injected with non-active and gelatine-stabilized colloidal gold processed by the method of DEL TURCO & PIETRA, *this process produces a* particle size of 250 ± 50 Å. Different methods of administering the gold colloid were employed.

Three rabbits were operated upon and the abdominal aorta was dissected free. A small catheter was introduced into the aorta through a puncture needle and brought down to the left common iliac artery, 0.5 ml amyl nitrite and 40 ml gold colloid at body temperature were then injected through the catheter. Twenty minutes later the aorta was ligated proximal to the catheter and the animal killed with an intravenous injection of air. Saline solution was injected through the plastic tube. The left common iliac vein was opened and perfusion of the left leg continued until the blood in the vein was replaced by saline solution. *Thereafter 500 ml isotonic glutaraldehyde 2.5 % in veronal acetate buffer* were continuously perfused through the leg for about 15 to 20 minutes at body *temperature. Samples of bone marrow* about one mm^3 in size were cut out from the leg for further fixation in osmic acid.

One rabbit was injected intravenously with 10 ml colloidal gold on three consecutive days. It was kept alive for 24 days in apparently good condition and then anaesthetized with phenobarbital when a catheter was introudeced into the aorta. The procedure for the preservation was thereafter the same as described.

The injection of the gold colloid in 5 rabbits was performed intravenously through the ear vein with 20 ml each day on two consecutive days without any obvious general effects. This was followed on the third day by a further 20 ml *colloidal gold under 40 mg phenobarbital/kg, immediately before* (10 ml) and during excision (10 ml) of the bone marrow sample. To obtain the sample the femur was split and chilled isotonic and buffered glutaraldehyde 2.5 % dripped directly onto the exposed marrow by the method described by ZAMBONI & PEASE (1961). After 5 minutes' drip the bone marrow was immediately placed and left for one hour in glutaraldehyde for further stabilization. Two animals were injected intravenously with a single dose of 50 ml of the colloid, they were severely affected and were excluded from further investigations. The last method



Fig 1 A sinusoid (S) with a sinusoidal lining cell (L_1) and surrounding bone marrow cells Gold particles lie in vacuoles (arrows) in the sinusoidal lining cell, part of the nucleus (N) Outside and close to the sinusoid, parts of cells (L_2, L_3) with cell structures similar to those of the sinusoidal lining cell Magnification ×9 000

was also used in 3 control rabbits although corresponding volumes of saline solution were given in place of the colloid

The glutaraldehyde stabilization having been completed the samples of bone marrow were placed in isotonic osmic acid 1 % buffered with veronal acetate for one hour, dehydrated in acetone and embedded in Vestopal. Thin sections were cut on a LKB microtome III and stained in uranylacetate and lead citrate The specimens were analyzed in a Siemens Electron Microscope AI at magnifications up to × 30 000

A control as to whether the preparation procedure for electron microscopy removed particles from the bone marrow sample was performed by the injection of one rabbit intravenously with active colloidal gold. Two bone marrow samples of larger size than those for electron microscopy were cut out and placed in a scintillation counter of the well crystal type Impulses from the bone marrow

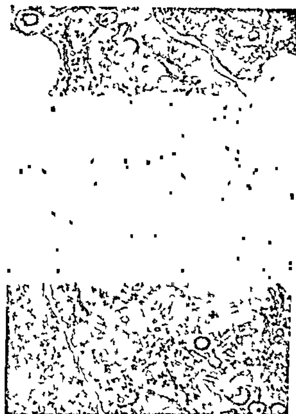


Fig 2



Fig 3

Fig 2 Part of a sinusoidal lining cell (L) A few gold particles are located in cytoplasmic vacuoles (arrows) easily differentiated from other cell particles Magnification ×25 500

Fig 3 Part of a sinusoidal lining cell Gold particles intimately attached to the plasma membrane (Cm) in a membranous invagination (arrow) and in cytoplasmatic vesicles together with thin filamentous material (V) One particle adheres to the cell membrane opposite the sinusoidal lumen (→→) Magnification ×25 000

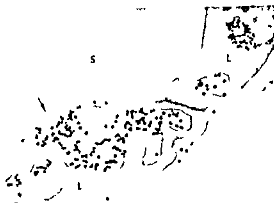


Fig 4 Two adjacent sinusoidal lining cells (L) constitute the thin wall of a sinusoid The sinusoidal lumen (S) has a deep invagination (arrow) gold particles located on both sides of the lower lining cell Magnification ×25 000



Fig 5 A megakaryocyte (M) lies outside the sinusoidal lining cell (L) Part of the nucleus of the lining cell (N) and colloidal gold particles accumulated in cytoplasmatic vesicles (V) A few colloidal particles are evident (→) in the narrow extracellular space between the lining cell and the megakaryocyte Two colloidal particles are present in the cytoplasmatic lumina of the demarcation membrane system (→→) Magnification ×15 500

samples were registered and the specimens placed in glutaraldehyde 2 5 % solution for one hour The activities in the glutaraldehyde solution and in the bone marrow specimens were then measured separately and compared with those from a control solution of glutaraldehyde The bone marrow specimens were then soaked in an osmic acid solution for one hour more, and measurements of the activities in the specimens and in the osmic acid solution were then performed

Results

The colloid particles processed by the method of DEL TURCO & PIETRA were uniform in most preparations with a diameter of about 250 Å Single particles of larger size (about 1 000 Å) could be observed in some tissue sections The particles were identified by their regular size and rounded shape as well as by



Fig 6

Fig 7

Fig 6 Two lining cells (L_1 L_2) separated by a sinusoidal lumen (S) A fat cell (F) lies in close connection with the lining cell L_1 An immature erythrocyte (E) appears to the upper right Colloidal gold particles located in the sinusoidal lumen and in invaginations of the cell surface of the lining cell L_1 (arrow) No colloidal particles or signs of phagocytosis of colloidal particles are evident in the fat cell Many particles present in membrane bound spaces of the lining cells Magnification $\times 11\,500$

Fig 7 Thrombocytic aggregation within a sinusoidal lumen Gold particles are located at the plasma membranes of the aggregated thrombocytes Magnification $\times 18\,000$

their high electron density, they were easily distinguishable from naturally occurring cell particles (Figs 1 2) The fraction of radiation activity released in the bone marrow specimens controlled by the use of ^{198}Au particles intravenously injected was negligible Only 0 4 per cent of the activity of the bone marrow specimen was released during one hour fixation of the bone marrow in glutaraldehyde and treatment with osmic acid solution for one hour freed an additional 0 05 per cent of the activity

A well preserved bone marrow specimen was obtained in the animals in which the bone marrow specimens were fixed in situ and glutaraldehyde was dripped onto the exposed marrow The preservation varied in animals with the legs perfused by intra arterial administration of glutaraldehyde The concentration of



Fig 8



Fig 9

Fig 8 Detail from a thrombocytic aggregation Colloidal particles situated externally to the cell membranes of the thrombocytes some particles lie within cytoplasmatic vacuoles (arrow) Magnification ×24 500

Fig 9 Wall of a sinusoidal lining cell (L) interrupted by a cytoplasmatic protrusion (p) from a megakaryocyte (M) Colloidal particles are phagocytized in the sinusoidal wall A particle arrow) is evident in a cytoplasmatic lumen in the protrusion Particles occur in the cytoplasmatic lumina in thrombocytes (T) within the sinusoidal lumen (S) Magnification ×7 500

gold particles in the bone marrow samples from rabbits injected intravenously was low after a single injection but was highest when 20 ml Au colloid was injected each day on three consecutive days Forty ml colloidal gold given intra-arterially as a single injection produced only a small number of gold particles in the bone marrow Regardless of the technique the gold particles appeared in only some of the sinusoids they were evident close to the plasma membrane of the sinusoidal lining cells facing the sinusoidal lumen, in invaginations of the plasma membranes, and enclosed in membrane bound spaces of these cells (Fig 3 The localization of the particles corresponded to that observed during the process of phagocytosis An interspace not less than 100 Å between the particle and the cross sectioned cell membrane was always present



Fig. 6. Two lining cells (L_1, L_2) separated by a sinusoidal lumen (S). A fat cell (F) lies in close connection with the lining cell L_1. An immature erythrocyte (E) appears to the upper right. Colloidal gold particles located in the sinusoidal lumen and in invaginations of the cell surface of the lining cell L_1 (arrow). No colloidal particles or signs of phagocytosis of colloidal particles are evident in the fat cell. Many particles present in membrane bound spaces of the lining cells. Magnification × 11 500.

Fig. 7. Thrombocytic aggregation within a sinusoidal lumen. Gold particles are located at the plasma membranes of the aggregated thrombocytes. Magnification × 18 000.

their high electron density, they were easily distinguishable from naturally occurring cell particles (Figs. 1, 2). The fraction of radiation activity released in the bone marrow specimens controlled by the use of ^{198}Au particles intravenously injected was negligible. Only 0.4 per cent of the activity of the bone marrow specimen was released during one-hour fixation of the bone marrow in glutaraldehyde, and treatment with osmic acid solution for one hour freed an additional 0.05 per cent of the activity.

A well preserved bone marrow specimen was obtained in the animals in which the bone marrow specimens were fixed in situ and glutaraldehyde was dripped onto the exposed marrow. The preservation varied in animals with the legs perfused by intra-arterial administration of glutaraldehyde. The concentration of



Fig 11 Part of a monocyte with a gold particle situated within an invagination of the cell membrane (arrow) A few particles are present in a vacuole Magnification ×12 500

cell structures could be observed in the extracellular space (Fig 12) Well-preserved cells were often present outside sinusoids of a lytic appearance, with or without gold particles, some of these had the same cytoplasmatic structures as the sinusoidal lining cells The lining cells often, through elongated adjacent cytoplasmatic processes, enclosed a multitude of bone marrow cells and reached other similar cells or sinusoids Sinusoidal cells with lytic changes but without gold particles were thus sometimes evident in the region of well preserved sinusoids containing gold particles (Fig 1) On the other hand, well preserved sinusoids without gold particles also lay beside lining cells with lytic changes and containing gold particles

The rabbit that survived for 24 days had gold particles in the sinusoidal cells as described earlier for the animals that lived only a short time No particles were evident in other cell elements of the bone marrow The particles had collected into large cytoplasmatic vesicles often containing a dark ground substance, many particles had lost their contours and were lumped together, sometimes in an almost structureless dense mass (Fig 13) The three animals used as controls had no gold particle like structures

Discussion

Colloidal gold particles were chosen from the many colloids available because these are easily identified in a tissue section, they have a well defined small particle size and colloidal ^{198}Au has been used extensively in human clinical bone marrow scanning (Engstedt et coll 1958, Larsson et coll 1960, 1964, Edwards et coll 1964, Kniseley et coll 1964, Kniseley 1972) It was important that the particles should be small as the smaller the particles the larger is the quantitative accumulation in the bone marrow after an intravenous injection (Dobson et coll 1949, Dobson & Jones 1952, Zilversmit et coll

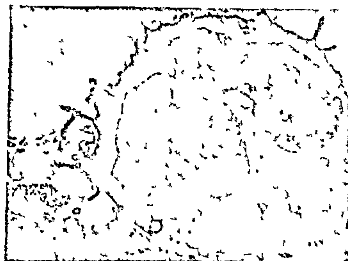


Fig. 10. Monocyte within a sinusoidal lumen. Gold particles adherent to the cell surface and phagocytized. Magnification × 13 000.

A few particles adhered to the cellular membrane opposite the sinusoidal lumen (Fig. 4). Particles were seldom present further outside the sinusoid among the haematopoietic cells of the bone marrow, and then only as scattered single particles. In three of 30 megakaryocytes counted, all situated outside and close to the sinusoidal wall, single gold particles were occasionally apparent, *a few of large size, the particles were located in the lumina of the demarcation* membrane system described by BEHNKE (1968) (Fig. 5). In spite of a great number of particles enclosed in the lining cells there were no signs of gold particles in the fat cells (Fig. 6) which appear in close connection with the sinusoids.

Many particles appeared external to the cell membranes in thrombocytic aggregations observed within the sinusoidal lumina (Fig. 7). Solitary particles *were evident in the vacuoles in the interior part of the thrombocytes*, no convincing morphologic signs of phagocytosis were however observed (Figs. 7, 8). Particles were evident in processes of megakaryocytic protoplasma *protruding into* the sinusoidal lumina (Fig. 9). The processes possibly represented thrombocytes in statu nascendi (DE BRUYN 1964, BEHNKE 1969). Of about 300 leukocytes examined (granulocytes, lymphocytes and plasma cells), often situated close to sinusoidal lining cells containing gold particles, none contained phagocytized gold particles. Of 48 monocytes counted, three had gold particles in membrane-bound spaces in the cytoplasma (Figs. 10, 11).

The membranes of many sinusoidal lining cells were ruptured and the cytoplasmatic structures were indistinct with areas of unidentifiable materials. These modifications of the *cell structures were interpreted as being due to lysis*. The membranous outer wall of a lining cell had sometimes partly disappeared and

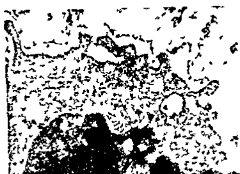


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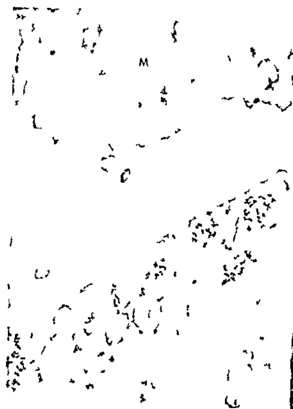


Fig. 12



Fig. 13

Fig. 12. Sinusoidal lining cell (L) with advanced lysis. Gold particles situated in mainly intact vacuoles. Well preserved cell (M) probably belongs to the myelopoiesis. Magnification ×18 500.

Fig. 13. Part of a lining cell (L) bounding a sinusoid (S). An early erythroid precursor (E) lies outside the sinusoid. Dark gold particle like structures and dark irregular masses appearing as lumped bold grains in cytoplasmatic vacuoles (V) in the lining cell. The bone marrow specimen was taken from a rabbit killed 24 days after an intravenous injection of colloidal gold. Magnification ×14 500.

1952). Single particles of larger size sometimes appeared in a tissue section but were too few to be of any importance in the interpretation of the observations.

In evaluating the distribution of colloidal particles in tissue sections the possibility arose that particles could have been released from the bone marrow specimens during the process of preservation. Measurements from experiments with active gold indicated however that only a small amount of activity was released during this process. The best preservation of the tissue was obtained when chilled glutaraldehyde was dripped onto the exposed bone marrow in situ. The variable preservation obtained by intra-arterial perfusion with glutaraldehyde may be due to some intravascular thrombosis or a vascular block due to swelling of the soft tissues in the bone channels (BOHMAN & MAUNSBACH 1970).

Despite a great number of particles adhering to the cell membranes of the sinusoidal lining cells or phagocytized by the lining cells, remarkably few particles were left in the sinusoidal lumina. With perfusion, particles not adhering to the cell membranes, and possibly also some of those that did adhere, could have been washed out from the sinusoids. In the method of continuous intravenous injection of colloidal gold the blood supply to the bone marrow was cut off just before the splitting of the femur. Blood is supplied to the bone marrow of the femur partly through vessels in the nutrient foramen and partly through minute vessels in the Haversian canals (BROOKES & HARRISON 1957, BRÅNEMARK 1961, ZAMBONI & PEASE, DE BRUYN et coll. 1970). It is probable that the short time necessary for splitting the femur was sufficient for the sinusoidal lining cells to phagocytize particles and thus remove them from the sinusoidal lumina.

Only a few of the sinusoids in the bone marrow are normally open for blood circulation, the conditions however change continually and new sinusoids will be opened for circulation while others will be closed (DOAN 1922, SABIN 1928, BOND et coll. 1962). This may explain why only a few sinusoids contained particles after a single injection. A repeat injection may open other sinusoids so that more sinusoidal lining cells will be able to phagocytize particles.

The interspace between adhered or enclosed gold particles and cellular membranes of the sinusoidal lining cells may be explained in different ways. It was not possible in this investigation to estimate whether the interspace depended on a structure belonging to the membrane or on an assumed coating of the gold particles. The coating of gold particles by stabilizing agents or plasma constituents after the intravenous injection of the colloid has previously been discussed (LAHR et coll. 1955, ZILVERSMIT et coll., KNISELEY et coll. 1948, HALPERN et coll. 1953, MCCORMICK et coll. 1954, MURRAY & KATZ 1955). An interspace to the cellular membranes of macrophages was earlier observed during phagocytosis of bacteria by these cells (BRAUNSTEINER et coll. 1960).

The great ability of the reticulo-endothelial cells to phagocytize particles is well known. Thus, where the bone marrow is concerned, accumulation of colloidal particles injected intravenously is to be expected primarily in the reticulo-endothelial cells, that is the sinusoidal lining cells. Under special conditions almost every cell may be able to phagocytize particles (LUBARSCH 1925) and phagocytosis can be demonstrated in most of the different cells of the bone marrow (JACOBSTHAL 1921, STRUMIA & BOERNER 1937, FEHER & KOMÁRI 1956, ZAMBONI & PEASE 1961). Among the cells of the circulating blood present in the sinusoids phagocytic properties are known in monocytes and neutrophilic granulocytes, discussed in terms of macro- and microphages by M[illegible] [illegible]NIKOFF 1887. Ph[illegible] [illegible] et coll. [illegible] (CLINE [illegible] mem-

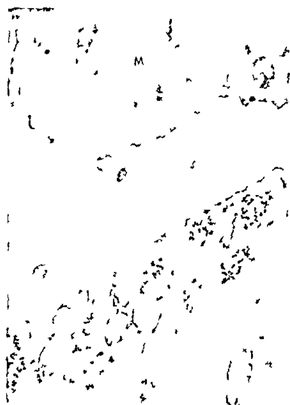


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difference between a sinusoidal cell or a 'fixed macrophage' will be only momentary

The conditions in the human bone marrow after an intravenous injection of colloidal radiogold are probably similar to those in the bone marrow of the rabbit The gold particle distribution presented here may thus reflect conditions during clinical bone marrow scanning The uptake of colloidal ^{198}Au observed in clinical examinations would therefore be a combined expression of the blood flow through the bone marrow and the ability of the sinusoidal lining cells in the bone marrow to phagocytize gold particles

Acknowledgements

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SUMMARY

The distribution of colloidal gold particles injected intravenously into the bone marrow of rabbits was investigated at ultrastructural level The accumulation of the particles revealed a high degree of selectivity for the sinusoidal lining cells and only scattered particles lay outside the sinusoids The uptake of colloidal gold in the bone marrow seems to be essentially a function of the blood flow and of the ability of the sinusoidal lining cells to phagocytize the colloidal particles

ZUSAMMENFASSUNG

Nach intravenoser Injektion von kolloidalen Goldpartikeln wurde deren Verteilung im [illegible] Es zeigte sich, [illegible] und nur ge[illegible]ten Die An[illegible]ung des kolloidalen Goldes hangt im wesentlichen vom Blutzufluss und von der Phagozytose der Auskleidezellen der sinoidalen Raume ab

RÉSUMÉ

Les auteurs ont etudie au niveau ultrastructural la distribution des particules d or colloidal dans la moelle osseuse apres injection intraveineuse sur des lapins L accumulation des particules a montre un haut degre de selectivite pour les cell[illegible] [illegible] à phagocyter les particules colloidales

branes (BLOOM et coll. 1955, BEHNKE 1968) and to phagocytize particles (SCHULTZ 1960, DAVID-FERREIRA 1964, MOVAT et coll. 1965, GLYNN et coll. 1965, 1966, MUSTARD & PACKHAM 1968).

Only a few gold particles appeared outside the sinusoid among the cells of the *myelo- and erythropoiesis*, despite many particles within the sinusoidal lumen or its lining cells. A different distribution of particles in the bone marrow was obtained after the intravenous injection of colloidal thorium dioxide (ZAMBONI & PEASE) but a similar distribution was observed after the injection of coal particles (HUHN & STEIDLE 1967). It would appear that the circulation in the bone marrow is a closed one as far as colloidal gold is concerned. A possible mechanism of transportation for the few particles that pass the sinusoidal wall is that particles are phagocytized and then transported by membrane flow in vacuoles through the lining cells (LEWIS 1931, PALADE 1960, BENNETT 1956). The few particles passing the sinusoidal wall are sometimes evident in the demarcation membrane system of the megakaryocytes, probably lodging there as a result of diffusion. A few gold particles were observed in some monocytes and thrombocytes of the circulating blood. What was remarkable was the absence of convincing signs of phagocytosis in the thrombocytes, in spite of many particles being attached to the cell surface. The phagocytic capacity of the reticulo endothelial system seemed as far as colloidal gold was concerned to be overwhelming in competing for the particles. As gold particles may still be evident in cytoplasmatic vacuoles in the lining cells 24 days after the intravenous injection, long-lasting retention of the particles in these cells appears to exist. Retention of particles by the reticulo-endothelial cells has previously been described by HAHN (1951) and by JUHLIN (1960).

The cells apparently undergoing lysis may be artefacts from the process of preservation. If this were so however a certain number of gold particles should be released during the process of preservation, this apparently failed to occur. Moreover, the sections indicated that these lytic cells were surrounded by intact cells. WEISS (1965, 1970), making the same observations concerning the lysis of sinusoidal cells, suggested that the sinusoids are in a state of continuous re-arrangement. This would also explain the occurrence of colloidal gold particles on both sides of a sinusoidal lining cell. No clear-cut distinction has been made in this investigation between sinusoidal cells' and reticular cells or 'fixed macrophages' of the bone marrow (BLOOM & FAWCETT 1968). Phagocytizing cells, similar to the sinusoidal cells, appear outside the sinusoids but only by means of serial sections is it possible to conclude whether these belong to a sinusoid or not. The explanation of WEISS that the bone marrow is in a state of continuous re-arrangement seems very likely according to the observations in this investigation. If the vascular pattern of the bone marrow is continuously being re-arranged the

Fresen O und Lierenfeld M Die Wirkung des Blutverlustes und des akuten Saponinversuches auf die Myelopoese des Kaninchens Virchows Arch path Anat 328 (1956), 273

Glynn M F, Movat H Z, Murphy E A and Mustard J F. Study of platelet adhesiveness and aggregation with latex particles J Lab clin Med 65 (1965), 179

— Herren R and Mustard J F Adherence of latex particles to platelets Nature 212 (1966), 79

Hahn P F Radioactive colloids in the treatment of lymphoid-macrophage diseases *In:* A manual of artificial radioisotope therapy, p 139 Edited by P F. Hahn Academic Press Inc New York 1951

Halpern B N, Benacerraf B and Biozzi G Quantitative study of the granulopectic activity of the reticuloendothelial system, effect of ingredients present in India ink and of substances affecting blood clotting in vivo or fate of carbon particles administered intravenously in rats, mice and rabbits [illegible]

Huhn D
Wirk

Jacobst
Leuk
Virchows Arch path Anat 234 (1921), 12

Juhlin L Retention of particles by the reticulo endothelial system Acta physiol scand 48 (1960), 78

Kaye G I and Pappas G D Studies on the cornea I The fine structure of the rabbit cornea and the uptake and transport of colloidal particles by the cornea in vivo J cell Biol 38 (1962) 457

Kniseley M H, Bloch E H and Warner L Selective Phagocytosis Kgl Danske Viden-

Lahr T N, Olsen R, (
^{198}Au ^{32}P and ^{90}Y, ir
45 (1955), 66

Larsson L-G, Engstedt L, Franzén S and Jonsson L Bone marrow scintigrams An in vivo study of the bone marrow reticulum in malignant bone marrow diseases Acta Un int Cancr 16 (1960), 1473

McCormick J B Miller G, Jaffe B and Seed L Tissue distribution of injected radioactive colloidal chromic phosphate ($CrP^{32}O_4$) Arch Path 58 (1954), 187

Moore R D, Mumaw V R and Schoenberg M D The transport and distribution of colloidal iron and its relation to the ultrastructure of the cell J Ultrastruct Res 5 (1961), 244

Movat H Z, Weiser W J, Glynn M F and Mustard J F Platelet phagocytosis and aggregation, J cell Biol 27 (1965), 531

REFERENCES

BEHNKE, O.: An electron microscope study of the megakaryocyte of the rat bone marrow. I. The development of the demarcation membrane system and platelet surface coat. J. Ultrastruct. Res. 24 (1968), 412.

— Electron microscopical observations on the surface coating of human blood platelets. J. Ultrastruct. Res. 24 (1968), 51.

— An electron microscope study of the rat megakaryocyte. 2. Some aspects of platelet release and microtubules. J. Ultrastruct. Res. 26 (1969), 111.

BENNETT, H. S.: The concept of membrane flow and membrane vesiculation as mechanisms for active transport and ion pumping. Biochem. Cytol. 2 (1956), 99.

BLOOM, G., GUSTAVSSON, K. H. and SVENSSON, Å.: On the reaction of the thrombocytes of submicroscopic particles in vivo. Acta haemat. 13 (1955), 57.

BLOOM, W. and FAWCETT, D. W.: Blood cell formation and destruction. *In*: A textbook of histology, p. 184. W. B. Saunders Company, London 1968.

BOHMAN, S. O. and MAUNSBACH, A.: Effects on tissue fine structures or variations in colloid osmotic pressure of glutaraldehyde fixativ. J. Ultrastruct. Res. 30 (1970), 195.

BOND, V. P., FLIEDNER, T. M., USENIK, E. and UPTON, D. V. M.: Early bone marrow hemorrhage in the irradiated dog. Arch. Path. 73 (1962), 13.

BRÅNEMARK, P. I.: Experimental investigation of microcirculation in bone marrow. Angiology 12 (1961), 293.

BRAUNSTEINER, H., FIBL, M. und PAKESCH, F.: Elektronenmikroskopische Beobachtungen über Bakterienphagozytose durch Makrophagen. Wien. Z. inn. Med. 41 (1960), 373.

BROOKES, M. and HARRISON, R. G.: The vascularization of the rabbit femur and tibiofibula. J. Anat. 91 (1957), 61.

CLINE, J. M., HANIFIN, J. und LEHRER, R. I.: Phagocytosis by human eosinophils. Blood 22 (1968), 922.

DAVID FERREIRA, J. F.: The blood platelet. Electron microscopic studies. Int. Rev. Cytol. 17 (1964), 99.

DE BRUYN, P. P. H.: The fine structure of the megakaryocyte or the bone marrow of the guinea pig. Z. Zellforsch. 64 (1964), 111.

— BREEN, P. C. and THOMAS, T. B.: The microcirculation of the bone marrow. Anat. Rec. 168 (1970), 55.

DEL TURCO, A. M. and PIETRA, R.: On a method of preparation of colloidal radiogold (^{198}Au). Centro di Studi Nucleari di Ispra (CNI) 31 (1960).

DOAN, C. A.: Capillaries of the bone marrow. Bull. Johns Hopk. Hosp. 33 (1922), 222.

DOBSON, E. L., GOFMAN, J. W., JONES, H. B., KELLY, L. S. and WALKER, L. A.: Studies with colloids containing radioisotopes of Yttrium, Zirconium, Colombium and Lanthanum. J. Lab. clin. Med. 34 (1949), 305.

— and JONES, H. B.: The behaviour of intravenously injected particulate material. Acta med. scand. (1952), Suppl. No. 273.

EDWARDS, C. L., ANDREWS, G. A., SITTERSON, B. W. and KNISELEY, R. M.: Clinical bone marrow scanning with radioisotopes. Blood 23 (1964), 741.

ENGSTEDT, L., FRANZÉN, S., JONSSON, L. and LARSSON, L. G.: In vivo localization of colloidal ^{198}Au intravenously injected in polycythemia vera. Acta radiol. 49 (1958), 66.

FEHÉR, L. und KOMÁRI, J.: Über Phagozytose der Leukocyten bei verschiedenen hämatologischen Erkrankungen. Folia haemat. 73 (1956), 301.

FLEICHER, J.: Das normale Knochenmarkbild des Kaninchens. Folia haemat. 19 (1962), 89.

Fresen O und Lierenfeld M Die Wirkung des Blutverlustes und des akuten Saponinversuches auf die Myelopoese des Kaninchens Virchows Arch path Anat 328 (1956), 273

Glynn M F, Movat H Z, Murphy E A and Mustard J F Study of platelet adhesiveness and aggregation with latex particles J Lab clin Med 65 (1965), 179

— Herren R and Mustard J F Adherence of latex particles to platelets Nature 212 (1966), 79

Hahn P F Radioactive colloids in the treatment of lymphoid macrophage diseases *In* A manual of artificial radioisotope therapy p 139 Edited by P F Hahn Academic Press Inc New York 1951

Halpern B N Benacerraf B and Biozzi G Quantitative study of the granulopectic [illegible]

[illegible]

Virchows Arch path Anat 234 (1921) 12

Juhlin L Retention of particles by the reticulo-endothelial system Acta physiol scand 48 (1960 78

Kaye G I and Pappas G D Studies on the cornea I The fine structure of the rabbit cornea and the uptake and transport of colloidal particles by the cornea in vivo J cell Biol 38 (1962) 457

Kniseley M H Bloch E H and Warner L Selective Phagocytosis Kgl Danske Videnskab Selskab Biol Skrifter 4 (1948) 7

Kniseley R M Marrow studies with radiocolloids Sem nucl Med 2 (1972), 71

Andrews G A Edwards C L and Tanida R Scanning of bone marrow in haematopoietic disorders *In* [illegible]

La[illegible]

Larsson L G Engstedt L Franzén S and Jonsson L Bone marrow scintigrams An in vivo study of the bone marrow reticulum in malignant bone marrow diseases Acta Un int Cancr 16 (1960) 1473

and Jonsson L Bone marrow scanning after intravenous injection of colloidal gold ^{198}Au *In* Medical radioisotope scanning Vol II p 193 IAEA Vienna 1964

Lewis W H Pinocytosis Bull Johns Hopk Hosp 49 (1931) 17

Lubarsch O Über Phagocytose und Phagocyten Klin Wschr 4 (1925), 1248

Metschnikoff E Sur la lutte des cellules de l organisme contre l invasion des microbes Ann Inst Pasteur 1 (1887) 321

McCormick J B Milles G Jaffe B and Seed L Tissue distribution of injected radioactive colloidal chromic phosphate ($CrP^{32}O_4$) Arch Path 58 (1954), 187

Moore R D Mumaw V R and Schoenberg M D The transport and distribution of colloidal iron and its relation to the ultrastructure of the cell J Ultrastruct Res 5 (1961), 244

Movat H Z Weiser W J Glynn M E and Mustard J F Platelet phagocytosis and aggregation J cell Biol 27 (1965) 531

MURRAY, I. M. and KATZ, M.: Factors affecting the rate of removal of gelatin stabilized radiogold from the blood. J. Lab. clin. Med. 46 (1955), 263.
MUSTARD, J. F. and PACKHAM, M. A.: *Platelet phagocytosis. Ser. Haemat. 1 (1968), 168.*
PALADE, G. E.: Transport in quanta across the endothelium of blood capillaries. Anat. Rec. 136 (1960), 189.
SABIN, F. R.: Bone marrow. Physiol. Rev. 8 (1928), 191.
SATERBORG, N.-E.: Mikrofordelningen av kolloidalt guld i benmargen efter intravenös injektion. (In Swedish.) Nord. Med. 85 (1971), 160.
SCHULTZ, H.: Über Phagocytose von kolloidalem SiO_2 in Thrombocyten. Zbl. allg. Path. path. Anat. 102 (1960), 319.
SHEPPARD, C. W., BUIST, E., HAHN, P. F. and GOODELL, J. P. B.: Studies of the distribution of intravenously administered colloidal sols of manganese dioxide and gold in human beings and dogs using radioactive isotopes. J. Lab. clin. Med. 32 (1947), 274.
STRUMIA, N. M. and BOERNER, F.: Phagocytic activity of circulating cells in the various types of leukemia. Amer. J. Path. 13 (1937), 335.
WEISS, L.: *The structure of bone marrow. Functional interrelationships of vascular and haematopoietic compartments in experimental hemolytic anemia.* J. Morph. 117 (1965), 467.
— *Transmural cellular passage in vascular sinuses of rat bone marrow.* Blood 36 (1970), 189.
WISSIG, S. L.: An electron microscope study of the permeability of capillaries in muscle. Anat. Rec. 130 (1958), 526.
VON HERRATH, E.: Die Morphologie des retothelialen System. Verh. dtsch. Ges. Path. 37. Tagung (1954), 13.
ZAMBONI, L. and PEASE, D. C.: The vascular bed of red bone marrow. J. Ultrastruct. Res. 5 (1961), 65.
ZILVERSMIT, D. B., BOYD, G. A. and BRUCER, M.: The effect of particle size on blood clearance and tissue distribution of radioactive gold colloids. J. Lab. clin. Med. 40 (1952), 255.

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BONE MARROW ABNORMALITIES AFTER PHENYLHYDRAZINE INDUCED HEMOLYSIS IN RABBITS

N E SATERBORG

A correlation between the location of colloid accumulating reticulo-endothelial cells and the hematopoietic activity of the bone marrow has been reported (ROOT et coll 1954 NELP et coll 1966, GREENBERG et coll 1966) At microscopy however a definite difference was observed in normal marrow of rabbits (SATERBORG 1974) Under pathologic conditions in man differences between the extension of colloid accumulating cells and hematopoiesis may occur (VAN DYKE et coll 1967 ROSENTHALL et coll 1969, HAUSER et coll 1970)

Hemolytic anemia and stimulation of the bone marrow may be induced by phenylhydrazine in animal experiments (CUSTER 1932, BODANSKY et coll 1932, JACOBSEN et coll 1956) Colloidal gold accumulates only in the patent sinusoids of the bone marrow The significance of the different cells in the fat marrow during its conversion to red hematopoietic marrow during phenylhydrazine stimulation may be elucidated by observing the location of the colloidal ^{198}Au

The bone marrow structure of the adult rabbit is fairly comparable to that of the adult human (VON HERRATH 1953, FLEICHER 1962) Yellow inactive (fat) bone marrow is in adult rabbits regularly found in the distal part of the tibia

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Material and Methods

Uptake of colloidal ^{198}Au in the bone marrow after phenylhydrazine injections. The uptake of colloidal ^{198}Au in the bone marrow of the tibia was quantitatively measured after intravenous administration in 18 phenylhydrazine treated rabbits and in 12 controls. The animals were given 2.5 mg phenylhydrazine hydrochloride daily. They were killed at varying intervals up to 45 days. The reticulocytes were counted in all animals immediately before they were killed and in four animals also at several times during the phenylhydrazine treatment. A tracer dose of 100 μCi ^{198}Au was injected into all phenylhydrazine treated rabbits and controls 90 min before killing. The tibiae of the animals were excised and dissected free from soft tissues. They were then divided into two parts of equal length. After liquefaction in concentrated HCl the uptake was measured in a well scintillation counter and the amount was expressed as a percentage of the ^{198}Au injected.

Microscopy. Eight rabbits were given daily hypodermic injections of 2.5 mg phenylhydrazine hydrochloride. Two rabbits were killed after 6 days, two after 10 days and four after 14 days. Twenty ml colloidal gold were injected intravenously daily for three days and the animals were killed with mebumal sodium and air insufflation two hours after the last injection. Six rabbits were used as controls. Two of them were injected with phenylhydrazine for 14 days but received no colloidal gold. The distribution of colloidal gold (20 ml daily for 3 days) was examined in two rabbits without previous phenylhydrazine treatment and in two rabbits after daily injections of physiologic saline for 14 days. The colloidal gold was processed by the method of DEL TURCO & PIETRA (1960) and contained 5 mg gold/ml. Phenylhydrazine hydrochloride was dissolved in distilled water to a 2.5 % solution. Bone marrow samples were taken from the proximal, middle and distal parts of the tibia in all animals. They were placed for 24 hours in alcohol-formalin (Methanol 850 g, Sol. formaldehyd. 100 g, Acid. acet. conc. 50 g) for fixation, paraffin embedded, sectioned and stained with hematoxylin-eosin or congo-corinth (GELLERSTEDT 1944).

Results

Uptake of colloidal ^{198}Au in the bone marrow after phenylhydrazine injections. Following 4 to 6 days of phenylhydrazine treatment the reticulocyte count was significantly raised and then continued to increase up to about 50 per cent. The uptake of ^{198}Au in the marrow of the tibia began to increase in some rabbits after 6 days of phenylhydrazine treatment and after 10 days it was elevated regularly (Fig. 1). After 10 days it was approximately three times higher than in un-

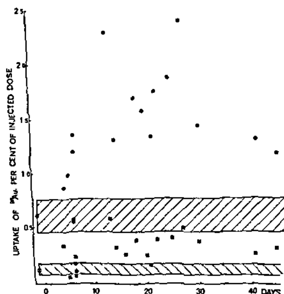


Fig 1 Uptake of ^{198}Au colloid in the proximal and distal parts of tibia of 18 phenylhydrazine treated rabbits ● = uptake in proximal tibia ■ = uptake in distal tibia The hatched areas mark the normal ranges ○ = mean uptake in proximal tibia □ = mean uptake in distal tibia in 12 untreated animals

treated animals The increase was similar in the proximal and the distal parts (Fig 2)

Microscopy A proximal hematopoietic zone, an intermediate zone with fat and red marrow and a distal zone with fat marrow was observed in the marrow of the tibia from a control rabbit In the phenylhydrazine treated animals it was evident even macroscopically that the red hematopoietic zone had expanded in

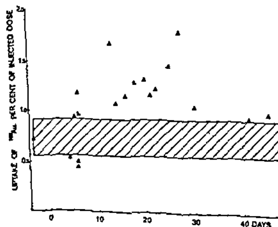


Fig 2 Uptake of ^{198}Au colloid in all bone marrow of tibia in 18 phenylhydrazine treated rabbits The hatched area marks the normal range △ = mean uptake in the tibia in 12 untreated animals

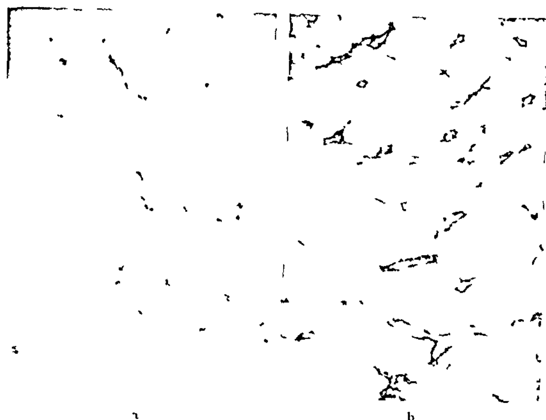


Fig 3 a) Bone marrow from the distal part of tibia of a normal rabbit Colloidal gold particles in the walls of a few capillary vessels ×100 b) Bone marrow from the distal part of tibia after phenylhydrazine treatment for 10 days Capillary dilatation and proliferation demonstrated by uptake of colloidal gold ×100

a distal direction during the phenylhydrazine treatment, in animals killed after a shorter period than five days this was however not encountered

Distended capillary vessels were evident at microscopy in animals killed after 6 and 10 days and the number of vessels had increased indicating hyperemia and proliferation (Fig 3) Colloid particles were concentrated to the endothelium of the patent vessels and were in the early stages of capillary proliferation located to singular fixed macrophages (Fig 4) All stages of capillary proliferation and expanding hematopoiesis occurred reticulum cells containing colloidal gold formed the capillary walls Undifferentiated reticulum cells were frequent close to but always outside the capillaries and always without colloid particles In the periphery of the expanding hematopoietic tissue there were many colloid accumulating vessels without surrounding hematopoiesis (Fig 5) In areas proximal or lateral to this peripheral zone the colloid accumulating vessels were surrounded by small colonies of granulocytopoietic cells lying beside the undifferentiated reticulum cells which were still frequent (Fig 6) In the proximal parts or in a



Fig 4 Fig 5

Fig 4 Bone marrow from the middle part of tibia after phenylhydrazine treatment for 16 days Colonies of cells belonging to the granulocytopoiesis Phagocytic reticulum cell with phagocytized colloidal gold (←+) Small vessel with colloidal gold aggregations outlining the capillary wall possibly representing an early stage of capillary proliferation (←↔) Larger capillary with the wall outlined by colloidal gold aggregations Outside the vessel an undifferentiated reticulum cell (←) ×380

Fig 5 Capillary in the fat marrow after phenylhydrazine treatment for 16 days Phagocytic reticulum cells The capillary wall is outlined by aggregations of colloidal gold Outside the vessel a single undifferentiated reticulum cell No surrounding erythro- or granulocytopoiesis ×500

rim around the fat marrow the colloid accumulating capillaries were surrounded both by granulopoietic and erythropoietic cells Signs of megakaryopoiesis also occurred and the capillaries appeared as bone marrow sinusoids as in the normal red marrow In the phagocytic reticulum cells, now appearing as sinusoidal lining cells, colloid particles were sometimes heavily concentrated and distinctly outlined the sinusoids (Fig 7) Undifferentiated reticulum cells could however still be found among the clusters of hematopoietic cells No observations indicated that the cells of the hematopoiesis were derived by differentiation of cells of the sinusoidal wall No colloidal gold was evident in the early or late cells of hematopoiesis or in the megakaryocytes Mitoses were sparse in the undifferentiated reticulum cells, but frequent among the cells of the granulocytopoiesis and erythropoiesis

Small areas of fat marrow, dilated vessels and bleeding into the bone marrow occurred in animals killed after 14 days Immature nucleated hematopoietic cells were frequently found in the dilated vessels



Fig 6

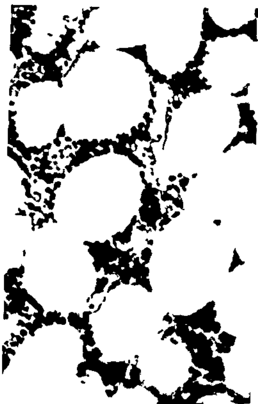


Fig 7

Fig 6 Bone marrow from the middle part of tibia after phenylhydrazine treatment for 27 days The capillary wall is outlined by aggregations of colloidal gold Outside the vessel a colony of cells containing several undifferentiated reticulum cells and some cells belonging to granulocytopoiesis × 500

Fig 7 A bone marrow sinusoid with the sinusoidal wall outlined by colloidal gold in the surrounding cells belonging to erythropoiesis or granulocytopoiesis (From a rabbit treated with phenylhydrazine for 6 days) × 90

In the periphery of the expanding hematopoiesis few cells had an appearance indicating lymphatic cells or monocytoid cells Such cells were however frequent in colonies of erythro- and granulocytopoietic cells

Discussion

Several methods are available for experimental transformation of fat bone marrow to red active marrow Bleeding has been used extensively since Neumann (1882) observed that red bone marrow extended in a peripheral direction following bleeding The hematopoiesis starts in the subendosteal parts of the fat marrow (Custer 1932) Even extensive bleeding does not always cause an extension of the red marrow however (Oehlbeck et coll 1932, Huggins &

BLOCKSOM 1936, BARTA 1963, KNISELEY et coll 1966) The temperature of the marrow is of significance for the transformation of the fat marrow to red active marrow (HUGGINS & BLOCKSOM 1936, MANIATIS et coll 1971) Low oxygen pressure also greatly stimulates the bone marrow (TRIBUKAIT 1963), and people living at high altitudes have a normoblastic bone marrow hyperplasia (MERINO & REYNAFARJE 1949) Factors related to hemoglobin cause stimulation of the bone marrow (ITAMI 1910, MCMASTER & HAESSLER 1921, SANCHEZ-MEDAL & LABARDINI 1968) and following bleeding in animals serum contains a factor that transforms fat marrow to erythropoietic marrow (BARTA & KADAS 1959)

Phenylhydrazine induced hemolysis transforms fat marrow to red marrow very efficiently Phenylhydrazine is a protoplasmic poison with many pharmacologic effects (BODANSKY et coll 1932, HUEPER 1936) Toxic liver effects were encountered in the present experiments It does not seem likely however that the liver injury caused a decreased uptake in the bone marrow A reduced colloid accumulation in the liver reticulum has been observed only after extensive destruction as in cirrhosis JACOBSEN et coll (1956) suggested that the liver injury after phenylhydrazine administration indirectly caused increased blood concentrations of erythropoietin The hemolysis is undoubtedly the main factor for the bone marrow stimulation Bleeding in the bone marrow occurred in animals given phenylhydrazine for more than 10 days, probably due to toxic effects of the phenylhydrazine upon the marrow

At microscopy a discrepancy between the extension of hematopoiesis and the occurrence of colloid accumulating reticulo-endothelial cells has been observed (SATERBORG 1974) This discrepancy seemed to be augmented after stimulation of the bone marrow The walls in the newly formed capillaries were obviously formed by fat marrow cells with phagocytic properties (phagocytic reticular cells of Maximow) and these capillaries finally appeared as bone marrow sinusoids Colonies of granulocytopoietic cells and, later on, also of erythropoietic cells appeared around the sinusoids

Intravenous injection of great amounts of gold colloid elucidated some consequences of the replacement of the fat marrow by active marrow Undifferentiated reticulum cells often occurred in the vicinity of newly opened capillaries and around these cells hematopoietic cells appeared These findings are in accordance with those by BARTA et coll (1963) and FITTING (1950) and support the theory that the hematopoietic cells normally derive from undifferentiated reticular stem cells (MAXIMOW 1927, BESSIS 1956, BLOOM & FAWCETT 1968 and others)

Stimulation of the bone marrow gives rise to hyperemia and proliferation of the capillaries resulting in formation of bone marrow sinusoids, the presence of which is a prerequisite for hematopoiesis (SABIN 1928, BARTA et coll 1963)

In resting fat marrow and during early stages of hematopoietic stimulation an uptake of colloid material may obviously occur in reticulum cells without concomitant hematopoiesis. This corresponds well with clinical observations, particularly in patients with anemia. By means of simultaneous use of ^{59}Fe and radioactive colloids it was thus found that the colloid accumulating cells sometimes had a larger extension in the bone marrow than the erythropoietic tissue (VAN DYKE et coll. 1967, KNISELEY 1972).

CAFFREY et coll. (1966) using ^{3}H-thymidine found that the 'reticular cells' had a low proliferating capacity which might support a concept of circulating stem cells for the hematopoiesis. In spite of the existence of transitional cell forms in bone marrow smears the possibility of blast cells derived from reticulum cells was not considered. The present investigation indicates however that at least some of the reticulum cells (the undifferentiated) may act as stem cells for hematopoiesis. As in previous reports (HARRIS et coll. 1963, KINDRED 1942), few mitoses were observed among the phagocytic or undifferentiated reticular cells. These cells may, however act as stem cells giving rise to a more rapidly multiplying compartment (MORSE et coll. 1970). TAVASSOLI & CROSBY (1970) considered that a fundamental histogenetic difference between erythropoietic and fat marrow existed and suggested that the conversion of fat to red marrow took place primarily by means of invasion of capillaries from the red marrow. Circulating stem cells may thus reach the fat marrow and initiate the transition to erythropoietic marrow. Circulating stem cells with the appearance of lymphocytes or monocytes have been described (CUDKOWICZ et coll. 1964, FORD 1966, VAN BEKKUM et coll. 1971, TYLER & EVERETT 1972, MALONEY & PATT 1972 and others). A few monocytoid cells and lymphocytes only were in the present investigation revealed in the early cell populations around the sinusoids. The role of the circulating stem cells is still much in dispute, some authors consider their significance for regeneration of hematopoietic tissue to be non essential (OSMOND et coll. 1966, FONG et coll. 1971). In the present investigation the dominating cells appearing early around the sinusoids were granulocytopoietic, between these and the undifferentiated reticulum cells other cells occurred giving the impression of intermediate forms.

A humoral factor is certainly involved in both the proliferation of bone marrow capillaries and the initiation of hematopoiesis. BARTA & KÁDAS (1959) were of the opinion that this factor was another one than erythropoietin, which however is generally believed to promote the differentiation of erythropoietic stem cells into normoblasts (MORSE et coll. 1970). It has also been reported that erythropoietin may stimulate vasoproliferation (FELEPPA et coll. 1971) and the production of stem cells (REISMANN & SAMORAPOOMPICHIT 1970). The significance of the colony stimulating factor (STOHLMAN & QUESENBERRY 1972) and of so

called granulopoietin (MORLEY et coll 1971) is uncertain and further investigations are needed

SUMMARY

The uptake of colloidal ^{198}Au in the proximal and distal parts of the tibia was measured in rabbits treated with daily subcutaneous injections of phenylhydrazine After 4 to 6 days the uptake increased after 10 days up to three times the values of normal rabbits The transition of the fat marrow to active marrow was elucidated by intravenously injected gold colloid demonstrating the patent bone marrow capillaries In the stimulated marrow the increased hematopoiesis started with hyperemia and capillary proliferation followed by hematopoietic cell colonies in the vicinity of newly formed sinusoids The granulocytic cell colonies appeared earlier than the erythropietic ones

ZUSAMMENFASSUNG

Die Aufnahme von kolloidalem ^{198}Au in den proximalen und distalen Teilen der Tibia wurde bei Kaninchen gemessen die taglich mit subkutanen Injektionen von Phenylhydrazin behandelt wurden Nach 4 bis 6 Tagen stieg die Aufnahme, nach 10 Tagen betrug sie den dreifachen Wert der normalen Tiere Die Umwandlung von fetthaltigem Knochenmark in aktives Knochenmark wurde durch intravenose Injektion von kolloidalem Gold untersucht wodurch die offenen Knochenmarkskapillaren dargestellt werden Beim stimulierten Knochenmark beginnt der Anstieg der Hamatopoiese mit einer Hyperamie und einer Proliferation der Kapillaren gefolgt von Zellkolonien in der Nahe der neugebildeten Sinusoide Die granulozytaren Zellkolonien traten fruher als die erythropoietischen auf

RÉSUMÉ

La fixation d or colloidal^{198} dans les parties proximales et distales du tibia a ete mesuree sur des lapins traites par des injections sous-cutanees quotidiennes de phenylhydrazine Apres 4 à 6 jours la fixation augmente apres 10 jours elle atteint le triple de sa valeur chez les lapins normaux La transition de la moelle graisseuse à la moelle active a ete elucidee grace à l or colloidal injecte par voie intraveineuse qui montre que les capillaires de la moelle osseuse sont ouverts Dans la moelle stimulee l augmentation de l hematopoiese a commence par une hyperemie et une proliferation capillaire suivie par l apparition de colonies de cellules hematopoietiques dans le voisinage des sinusoides nouvellement formes Les colonies de cellules granulocytaires sont apparues plus tôt que les cellules erythropoietiques

REFERENCES

BARTA I Hamatologie und Klinik des Fettmarkes Folia haemat [illegible]

[illegible]

BESS [illegible] Cytology of the blood and blood [illegible] York 1956

BLOOM W and FAWCETT D W Blood cell formation and destruction *In* A textbook of histology, p 184 W B Saunders Company, London 1968

BODANSKY M, MARR W L and BRINDLEY P An experimental study of the action of phenyl hydrazine hydrochloride and acetylphenylhydrazine (Pyrodin), with reference to their use in the treatment of polycythemia vera Amer J clin Path 2 (1932), 391

CAFFREY R W, EVERETT N B and RIEKE W O Radioautographic studies of reticular and blast cells in the hematopoietic tissues of the rat *Anat Rec 155 (1966), 41*

CUDKOWICZ G, UPTON A C, SMITH L H, GOSSLEE D G and HUGHES W L An approach to the characterization of stem cells in mouse bone marrow Ann N Y Acad Sci 114 (1964), 571

CUSTER R P Studies on the structure and function of bone marrow J Lab clin Med 17 (1932), 951

DEL TURCO A M and PIETRA R On a method of preparation of colloidal radiogold (^{198}Au) Centro Di Studi Nucleari Di Ispra (CNI) 31 (1960)

FELEPPA A E, MEINECKE H A and McCLUSKEY R S Studies of transplanted hemopoietic tissue utilizing a modified Algire back chamber I Vasoproliferation following erythropoietin stimulation Scand J Haemat *8 (1971), 86*

FITTING W Über die Bedeutung histologischer Knochenmarkuntersuchung Klin Wschr 28 (1950), 783

FLEISCHER J Das normale Knochenmarkbild des Kaninchens Folia haemat 19 (1962), 89

FONG P L, MALONEY M A and PATT H M Origin of repopulating cells in a mechanically depleted medullary cavity as determined by studies with marrow transplants Blood 37 (1971), 413

FORD C E Traffic of lymphoid cells in the body *In* Ciba Foundation Symposium on the Thymus, p 131 Edited by G E W Wolstenholme and R Porter Churchill London 1966

GELLERSTEDT N Eine neue Methode fur Knochenmarkfärbung an Gewebeschnitten Upsala Lak -Foren Forh *49 (1944)*, 447

GREENBERG M L, ATKINS H L and SCHIFFER L M Erythropoietic and reticuloendothelial function in bone marrow in dogs Science 152 (1966), 526

HARRIS P F HAIGH G and KUGLER J H Observations on the accumulations of mononuclear cells and the activities of the reticulum cells in bone marrow of guinea pigs recovering from whole body irradiation Acta haematol 29 (1963) 166

HAUSER W ATKINS H L and RICHARDS P Five years experience with ^{99m}Tc sulfur colloid N Y J Med 70 (1970) 848

HUEPER W C Pathological studies on the organic effects of various hydrazine derivatives J industr Hyg 18 (1936) 17

HUGGINS C and BLOCKSOM B H Changes in outlining bone marrow accompanying a local increase of temperature within physiological limits J exp Med 64 (1936) 253

ITAMI S Weitere Studien uber Blutregeneration Naunyn Schmiedeberg s Arch exp Pathol Pharmah 62 (1910), 104

JACOBSEN E M DAVIS A K and ALPEN E L Relative effectiveness of phenylhydrazine treatment and hemorrhage in the production of an erythropoietic factor Blood 11 (1956), 937

KINDRED J E A quantitative study of the hematopoietic organs of young albino rats Amer J Anat 71 (1942) 207

KNISELEY R M Marrow studies with radiocolloids Sem nucl Med 2 (1972) 71

— ANDREWS G A TANDIDA R EDWARDS C L and KYKER G C Delineation of active marrow by whole body scanning with radioactive colloids J nucl Med 7 (1966), 575

MALONEY M A and PATT H M Migration of cells from shielded to irradiated marrow Blood 39 (1972), 804

MANIATIS A, TAVASSOLI M and CROSBY W H Factors affecting the conversion of yellow to red marrow Blood 37 (1971), 581

MCMASTER P D and HAESSLER H The factor determining the spread of red marrow during anemia J exp Med 34 (1921) 579

MAXIMOW A Lymphoides oder reticulares Gewebe *In* Handbuch der mikroskopischen Anatomie des Menschen, S 335 Herausgegeben von W von Mollendorff J Springer, Berlin 1927

MERINO C R and REYNAFARJE C Bone marrow studies in the polycythemia of high altitudes J Lab clin Med 34 (1949) 637

MORLEY A, RICKARD K A, HOWARD D and STOHLMAN F Studies on the regulation of granulopoiesis IV Possible humoral regulation Blood 37 (1971), 14

[illegible]

bone marrow organ J nucl Med 7 (1966), 366

NEUMANN E Das Gesetz der Verbreitung des gelben und roten Markes in den Extremitaten knochen Zbl med W [illegible] 19 1909 [illegible]

C [illegible]

C [illegible]

unilateral limb shielding on the haemopoietic response of the guinea pig to gamma [illegible]

REI [illegible]

RO [illegible]

Ro [illegible]

SABIN F R Bone marrow Physiol Rev 8 (1928), 191

SÁNCHEZ MEDAL L and LABARDINI J Hemolysis and erythropoiesis III The effect of hemolysis and hemolysates on erythropoiesis Ann N Y Acad Sci 149 (1968), 377

STOHLMAN F and QUESENBERRY P J Colony stimulating factor and myelopoiesis Blood 39 (1972) 727

SÄTERBORG N E Accumulation of colloidal ¹⁹⁸Au in normal bone marrow of rabbits Microscopic and autoradiographic investigations Acta radiol Ther Phys Biol 13 (1974), 205

TAVASSOLI M and CROSBY W H Bone marrow histogenesis A comparison of fatty and red marrow Science 169 (1970), 291

TRIBUKAIT B Experimentelle Untersuchungen zur Regulation der Erythropoiese unter besonderer Berucksichtigung der Bedeutung des Sauerstoffs Acta physiol scand (1963) Suppl No 208

TYLER R W and EVERETT N B Radioautographic study of cellular migration using parabiotic rats Blood 39 (1972), 249

VAN BEKKUM D W, VAN NOORD M J, MAAT B and DICKE K A Attempts at identification of hemopoietic stem cell in mouse Blood 38 (1971), 547

van Dyke D., Shkurkin C., Price D., Yano Y. and Anger H. O.: Differences in distribution of erythropoietic and reticuloendothelial marrow in hematologic disease. Blood 30 (1967) 364.

von Herrath F.: Die Morphologie des Retothelialen Systems. Verh. dtsch. Ges. Path. 37. Tagung (1953), 13.

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BLOOD LYMPHOCYTES AFTER RADIATION THERAPY OF CARCINOMA OF PROSTATE AND URINARY BLADDER

HENRIC BLOMGREN, JERZY WASSERMAN and BO LITTBRAND

Local radiation therapy may result in a decreased number of lymphocytes in the peripheral blood This side effect of the treatment has been extensively investigated in patients with mammary carcinoma receiving local irradiation (MEYER & SAYRE 1970, THOMAS et coll 1971, MCCREDIE et coll 1972, STJERNSWARD et coll 1972, GLAS & WASSERMAN 1974, BLOMGREN et coll 1974)

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Fal [illegible] and its possible analogue in mammals, B cells (for a review of T and B cells see ROITT et coll 1969) Since the peripheral blood lymphocyte population represents a mixture of both T and B lymphocytes and possibly also other types of lymphocytes, attempts have been made to investigate whether the lymphopenia induced by local irradiation is due to a reduction of T or B or both cell types Using appropriate in vitro markers for human T and B lymphocytes it was recently observed that B lymphocytes are reduced to a higher extent than T lymphocytes following local irradiation of patients with mammary carcinoma BLOMGREN et coll) In vitro responsiveness of the cells to phytohaemagglutinin PHA was much less affected than their response to purified protein derivate

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of tuberculin (PPD) which was strongly suppressed (GLAS & WASSERMAN, BLOMGREN et coll.)

The present investigation was undertaken to determine whether there is a similar change of the peripheral blood lymphocyte population following local irradiation of patients with bladder or prostatic carcinoma as in those with mammary carcinoma. This question is of interest since these two groups of patients receive radiation to completely different parts of the body. It may be mentioned that the thymus, in irradiated breast carcinoma, is partly or completely included within the irradiated field.

Material and Methods

The material consists of nineteen patients, 15 males and 4 females between 52 and 75 years old, attending Radiumhemmet for treatment of prostatic or urinary bladder carcinoma. All patients received external irradiation. Four patients were irradiated by a ^{60}Co unit with 100 cm source to skin distance, and fifteen patients by a 6 MV linear accelerator. The entire small pelvis was exposed to irradiation using two oblique anterior beams with wedge filters and a direct posterior beam. Each patient was dose-planned individually. Patients with urinary bladder carcinoma received various doses of irradiation depending on the stage of the tumour and whether surgery was planned after irradiation. Some patients received 3 600 rad, 12 × 300 rad in four weeks, some received 6 400 rad, 32 × 200 rad in eight weeks with two weeks of rest when half the dose was given, some received 8 400 rad, 3 × 100 rad daily × 28 in eight weeks with two weeks of rest in the middle of the treatment. Patients with prostatic carcinoma received a total tumor dose of 5 400 rad, 30 × 180 rad in six weeks.

Separation of peripheral blood leukocytes. Two methods were employed. (1) Venous blood was drawn in heparinized syringes. The lymphoid cells were separated by centrifugation on a Ficoll Isopaque gradient (Ficoll. Pharmacia Fine Chemicals, Inc. Stockholm, Sweden. Isopaque. Nyegaard, Oslo, Norway) as detailed by others (JONDAL et coll. 1972). The leukocyte suspensions were washed once by centrifugation, resuspended in Eagle's Minimal Essential Medium supplemented with Earle's salts (MEM) and counted in a Burker chamber after crystal violet staining. These crude populations of lymphoid cells were used for lymphocyte stimulation tests and in the first set of tests were also used to examine the frequency of rosette forming cells. (2) Venous blood was collected in glass beakers without heparin. The blood was defibrinated by agitation of glass pearls present in the beakers. The leukocytes were separated from the erythrocytes by sedimentation at unit gravity after addition of a gelatin

Table I

Number of lymphocytes and frequency of E and EAC rosette forming cells in the peripheral blood of patients with prostatic or urinary bladder carcinoma before and after irradiation The frequency of rosette forming cells was established in nonpurified preparations of lymphoid cells Ten patients were examined The differences of the values obtained before and after were calculated for each patient separately The mean values of these differences ± SE are presented Probability values as calculated by Student's t test are also indicated

	Mean ± SE		
	Before	After	Difference
Cell number per μl of blood	1802 ± 266	788 ± 159	−1021 ± 315 $p < 0.025$
EAC cells (%)	46 4 + 6 9	24 3 ± 3 6	−22 3 ± 3 6 $p < 0.001$
E cells (%)	36 3 ± 7 3	42 6 ± 4 1	+6 3 ± 8 8 $p < 0.5$

solution as described in detail by COULSON & CHALMERS (1964) The leukocytes were then suspended in 10 ml of trisbuffered Hanks balanced salt solution supplemented with 10 % autologous serum and containing 0 4 g of iron powder The tubes were then incubated at 37° C for 30 min and rotated every 5th minute Phagocytic cells were then removed by a magnet (LUNDGREN et coll 1968) This was followed by centrifugation through a layer of Ficoll Isopaque Details of these procedures have been given before (BLOMGREN et coll) These highly purified lymphocyte suspensions were used to examine the frequency of various rosette forming cells

Rosette formation of lymphoid cells Human T lymphocytes adhere sheep erythrocytes to their cell surface in vitro in an immunologically nonspecific way whilst B lymphocytes do not (BRAIN et coll 1970, BRAIN & GORDON 1971, JONDAL et coll) The latter cells on the other hand have membrane bound receptors for the activated complement factor C 3 (BIANCO et coll 1970, MICHLMAYR & HUBER 1970, DUKOR et coll 1971, JONDAL et coll) The frequency of lymphoid cells forming rosettes with sheep erythrocytes (E cells) and those forming rosettes with sheep erythrocytes coated with specific rabbit antibodies and complement (EAC cells) were determined in crude lymphoid cell suspensions and in highly purified preparations of lymphocytes The methods have recently been described (BLOMGREN et coll)

Table 2

PHA response of lymphocytes from patients before and after irradiation. The PHA responses expressed as cpm are related to the mean responses of lymphocytes from two control subjects tested at the same time. Ten patients were tested. Their lymphocyte counts and frequency of T and EAC cells are presented in Table 1. The difference between the values obtained before and after irradiation are calculated as described in Table 1

Mean response in per cent of controls ± SE		
Before	After	Difference
8[illegible].1 ± 11.5	72.8 ± 11.[illegible]	−11.[illegible] ± 11.8 p > 0.[illegible]

Lymphocyte stimulants. Phytohaemagglutinin (PHA, Bacto phytohaemagglutinin M, Difco Lab., Detroit, Mich., USA). The contents of commercially available vials of PHA were dissolved in 5.0 ml of MEM. The cells were stimulated by PHA at a final concentration of 3.0 %. Purified protein derivate of tuberculin (PPD tuberculin, RT 23, Statens Seruminstitut, Copenhagen, Denmark).

Lymphocyte culture conditions. 0.5 × 10 lymphoid cells were cultured in screw cap glass tubes containing 1.0 ml of MEM with 10 % of human serum (HS) from AB, Rh neg. donors, 100 units of penicillin and 150 μg of streptomycin per ml. The HS was decomplemented by heating at 56° C for 30 min and stored at −20° C before use. The lymphocytes were stimulated by PHA or varying amounts of PPD. Control cultures received no stimulants. The cells were cultured for 5 days at 37° C in a humified 5 % CO_2 air atmosphere. Twentyfour hours before termination of the cultures each tube received 0.1 ml of MEM containing 0.4 μCi of ^{14}C thymidine (Radiochemical Center, Amersham, England. Specific activity 54 mCi/mM). The cultures were completed by centrifugation in the cold, washed twice in a balanced salt solution by centrifugation and precipitated twice in trichloroacetic acid. The precipitates were then dissolved in Soluene and the contents of the tubes transferred to vials containing scintillation fluid. Details of these procedures have been given by GLAS & WASSERMAN. Activity expressed as counts per minute (cpm) was recorded by a Packard Scintillation Counter Model 3380.

General design of the tests. Lymphocytes were obtained from the patients within one week before beginning of irradiation and within three weeks after its completion and before any other kind of specific therapy was started. As

Table 3

Number of lymphocytes and frequency of E and EAC rosette forming cells in the peripheral blood of patients with prostatic or urinary bladder carcinoma before and after irradiation The frequency of rosette forming cells was established in purified preparations of lymphoid cells Eight patients were examined The difference between the values obtained before and after irradiation are calculated as described in Table 1

	Mean ± SE		
	Before	After	Difference
Cell number per µl of blood	2085 ± 246	615 ± 110	−1470 ± 315 $p < 0\ 005$
EAC cells (%)	21 4 ± 15 9	15 9 ± 2 3	−5 4 ± 2 3 $p < 0\ 05$
E cells (%)	49 8 ± 2 9	51 6 ± 3 4	+1 4 ± 4 4 $p > 0\ 5$

discussed previously the degree of lymphocyte stimulation by nonspecific mitogens varies extensively from time to time although the determinations are performed using the same healthy cell donor and the experimental conditions are kept strictly constant (BLOMGREN et coll) The reason for this inter-experimental variability is unknown, but it is probably not due to changes of the peripheral blood lymphocyte population of the cell donor To overcome this variability the PHA responses of the lymphocytes from two healthy control persons were always tested every time a patient's lymphocytes were stimulated with the same mitogen The lymphocytes from one healthy control were stimulated with various doses of PPD every time a patient's lymphocytes were exposed to this agent The same control persons were used throughout this investigation

All cultures were set up in duplicate The background incorporations of ^{14}C-thymidine obtained in control cultures without stimulants were deduced from that obtained in PHA or PPD stimulated cultures. The degree of stimulation of the patient's lymphocytes were related to the mean stimulation obtained in the cultures from the respective control subject and expressed as per cent The degree of stimulation, expressed as cpm of the lymphocytes from the control subject exposed to PHA, varied from 25 000 to 80 000 The PPD response varied fram 15 000 to 30 000

The rosette forming tests are highly accurate in terms of frequency of E and EAC rosette forming cells in the peripheral blood lymphocyte population of the same healthy control subject tested at various times (BLOMGREN et coll) To

Table 4

Frequency of E and EAC rosette forming cells in purified lymphocyte preparations from a control subject tested on three occasions at 2–3 month intervals. These tests were performed parallel with those presented in Table 3

	1	2	3
EAC cells (%)	14	14	13
E cells (%)	53	62	51

further strengthen this finding the lymphocytes from one control were tested parallel with those from the patients.

Results

PHA response and frequency of E and EAC rosette forming cells in nonpurified lymphoid cell populations. The peripheral blood lymphocyte population in 10 patients was first established with regard to cell number, cellular composition and relative PHA response. Three of these patients were treated for prostatic carcinoma and seven for urinary bladder carcinoma. Two received a total tumor dose of 8 400 rad, three 5 400 rad and five 3 600 rad. The results obtained from these patients are pooled.

The number of lymphocytes in the peripheral blood was reduced to less than 50 per cent after completion of irradiation (Table 1). In nonpurified lymphoid cell preparations the frequency of cells forming EAC rosettes was found to decrease significantly following irradiation whereas the E rosette forming cells exhibited a slight, but not significant relative increase, the relative response of the cells to PHA did not change significantly (Table 2).

PPD response and frequency of E and EAC rosette forming cells in purified lymphocyte populations. Nine patients were examined. Four of them were treated for prostatic carcinoma and five for bladder carcinoma. Four received a total tumor dose of 6 400 rad, four 5 400 rad and one 3 600 rad.

The number of lymphocytes in the peripheral blood was reduced to approximately 30 per cent following irradiation (Table 3). The frequency of EAC rosette forming cells in purified lymphocyte preparations was found to decrease significantly whilst the frequency of E rosette forming cells did not change markedly. Purified blood lymphocyte preparations from a healthy control were examined in parallel experiments with regard to frequency of E and EAC rosette

Table 5

PPD response of lymphocytes from patients before and after irradiation The PPD responses, expressed as cpm are related to the response of lymphocytes from one control subject tested at the same time Seven [illegible] *'e 3* [illegible] *able* [illegible] *fore*

The mean value of these determinations, expressed as per cent ± SE are presented

PPD concentration μg/ml	Mean ± SE			Per cent of original value
	Before	After	Difference	
100	59 6 ± 21 4	13 1 ± 4 5	−46 5 ± 19 6 p < 0 1	34 7 ± 11 5 p < 0 005
10	93 8 ± 41 7	28 8 ± 9 1	−64 9 ± 34 8 p < 0 2	59 8 ± 21 9 p < 0 2
1 0	109 5 ± 59 8	36 8 ± 21 6	−72 8 ± 39 5 p < 0 2	84 6 ± 48 8 p > 0 5

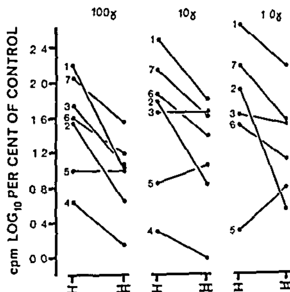
forming cells The frequency of these cells did not change markedly when tested at 2 to 3 month intervals (Table 4)

The response of lymphocytes to various concentrations of PPD in vitro was also tested before and after irradiation The mean PPD responses of seven patients decreased following irradiation (Table 5) A statistically significant decrease was only observed when the cells were exposed to 100 μg of PPD An extensive variability of the responses before irradiation occurred (Figure) It may be seen that there was a decrease of the PPD response in 6 out of 7 tested patients after irradiation In general, lymphocytes from patients with a high PPD response before irradiation exhibited the *strongest relative decrease* afterwards

Discussion

Several authors have stated that local irradiation of mammary carcinoma induces a lymphopenia in the peripheral blood (MEYER & SAYRE, THOMAS et coll, MCCREDIE et coll, STJERNSWARD et coll, GLAS & WASSERMAN, BLOMGREN et coll) A similar lymphopenia seems to develop in patients receiving local irradiation to other parts of the body, although this is less extensively established (THOMAS et coll, MCCREDIE et coll) Following local irradiation of breast carcinoma lymphocytes having receptors for C'3, presumably mainly B lymphocytes, are reduced to a higher extent than lymphocytes binding sheep erythrocytes to their cell surface, T cells (BLOMGREN et coll) No conclusive data

In vitro response of lymphocytes to various concentrations of PPD before (I) and after (II) local irradiation of the pelvic region. The responses are expressed as $\log_{10}$ per cent of the response of lymphocytes from a control subject. Seven patients were tested. Their values are illustrated separately and numbered from 1—7. The lymphocytes from patient 4 were not stimulated by 1.0 µg of PPD per ml and hence are not illustrated. Patients 3, 4, 5 and 6 received a total tumour dose of 6400 rad and patients 1, 2 and 7 received 5400 rad.



were obtained as to the response of the lymphocytes to PHA in vitro following irradiation, but the in vitro response of the cells to PPD decreased significantly (Glas & Wasserman).

Two groups of patients were examined before and after radiation therapy. Both groups were heterogenous with regard to the age and sex of the patients, type of tumour and radiation dose delivered. It is evident that a significant lymphopenia developed in the peripheral blood of both groups of patients following irradiation. Counts of E and EAC rosette forming cells demonstrated that the frequency of the latter cells was reduced significantly whereas the proportion of E cells was not. The failure to demonstrate a significant increase of E rosette forming cells might have purely methodologic reasons, the reproducibility of the E rosette test being not so high as that of the EAC rosette test. In the first group of patients, the frequency of E and EAC rosette forming cells in non purified preparations of lymphoid cells was examined, in the second group highly purified lymphocyte suspensions were used. The results obtained from the latter tests probably only reflect changes of the frequency of T and B lymphocytes, whereas the results of the nonpurified are more difficult to interpret since human monocytes may also form EAC rosettes under the experimental conditions used and monocyte rosettes might sometimes be taken for lymphocyte rosettes (Lay & Nussenzweig 1968). The present results in patients receiving irradiation to the pelvic region are in agreement with those obtained in patients with irradiated carcinoma of the breast. It is of interest that similar changes of the proportion of B and T cells seem to develop in the blood of patients receiving therapy with cytostatic drugs (Sen & Borella 1973).

Although the cellular composition of the lymphocyte population was observed to be significantly changed following irradiation, the relative response of the cells to PHA was not markedly changed This is in agreement with previous reports that no evident change of PHA response could be observed after irradiation of breast carcinoma (BLOMGREN et coll) Radiation induced decrease of the PHA response has been reported however (MILLARD 1965, THOMAS et coll , STJERNSWARD et coll) whereas others have noticed an increase (MCCREDIE) Since there is evidence that PHA stimulates both B and T cells to increased DNA synthesis, at least when they are kept in a mixture (PHILLIPS & ROITT 1973), it is not surprising that a change of the T B cell ratio does not result in any marked change of the PHA responsiveness

The present results indicate that irradiation of the pelvic region decreases the response of the patient's lymphocytes to PPD in vitro The type of human lymphocyte triggered by PPD is not known In the mouse however there is strong evidence that low concentrations of PPD act as an antigenic stimulus for presensitized T cells, whereas higher concentrations activate B lymphocytes in a completely nonspecific fashion (SELZER & NILSSON 1972, NILSSON et coll 1973) The results also indicate that the response of lymphocytes to both high and low concentrations of PPD decrease after pelvic irradiation At present it is not possible to give any explanation for the impaired PPD response However, the results are in agreement with those obtained in mammary carcinoma (GLAS & WASSERMAN)

In conclusion, local irradiation of the pelvic region induces the same types of changes of the peripheral blood lymphocyte population as local irradiation of the thoracic region, including the thymus, in mammary carcinoma

Acknowledgements

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SUMMARY

The *peripheral blood* lymphocyte population in patients with prostatic and urinary bladder carcinoma was examined before and after local radiation therapy The number of blood lymphocytes after therapy was reduced to 30 to 50 per cent The frequency of lymphocytes bearing membrane bound *receptors* for activated complement thymus independent cells decreased significantly whereas the frequency of lymphocytes binding sheep erythrocytes, thymus-dependent cells, remained essentially the same The relative response of the lymphocytes to PHA was *unchanged* whereas their stimulation by PPD in vitro was significantly impaired following irradiation.

ZUSAMMENFASSUNG

Die periphere Blutlymphozyten Population von Patienten mit einem *Prostata- oder* Blasen-Karzinom wurde vor und nach lokaler Strahlentherapie untersucht Die Zahl der Blutlymphozytes nach der Therapie war auf 30—50 % vermindert Die Frequenz der Lymphozyten, die Membran-gebundene Rezeptoren fur aktiviertes Komplement besitzen, — die Thymus-unabhangigen Zellen — viel signifikant, wahrend die Frequenz der *Schaferythrozyten bindenden Lymphozyten,* — der Thymus-abhangiger Zellen —, im wesentlichen unverandert blieb Die relative Antwort der Lymphozyten auf FHA war unverandert, wahrend die Stimulation auf PPD in vitro nach Bestrahlung signifikant herabgesetzt war

RÉSUMÉ

La population lymphocytaire du sang périphérique chez des malades atteints de cancer de la prostate et de la vessie a été examinée avant et après traitement local par les radiations Le nombre des lymphocytes sanguins est réduit après le traitement de 30 à 50 % La fréquence des lymphocytes portant sur leur membrane des récepteurs pour le complément *activé, qui sont des cellules thymo-indépendantes, a diminué de façon significative alors* que la fréquence des lymphocytes ayant des récepteurs pour les érythrocytes de mouton, qui sont des cellules thymo-dépendantes est restée dans l'ensemble la même La réponse relative des lymphocytes à la PHA n'a pas été modifiée alors que leur stimulation par la PPD in vitro a été significativement diminuée apres l'irradiation

REFERENCES

BIANCO C, PATRICK R and NUSSENZWEIG V A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes I Separation and characterization J exp Med 132 (1970), 702

BLOMGREN H, GLAS U, MELÉN B and WASSERMAN J Blood lymphocytes after radiation therapy of mammary carcinoma Acta radiol Ther Phys Biol 13 (1974), 185

— — and WILLETTS W A Rosette formation by peripheral lymphocytes Clin exp Immunol 8 (1970), 1

BRAIN P and GORDON J Rosette formation by peripheral lymphocytes II Inhibition of these phenomena Clin exp Immunol *8 (1971), 441*

COULSON A S and CHALMERS G G Separation of viable lymphocytes from human blood Lancet 1 (1964), 468

DUKOR P, BIANCO C and NUSSENZWEIG V Bone marrow origin of complement receptor lymphocytes Europ J Immunol 1 (1971), 491

GLAS U and WASSERMAN J Effect of radiation treatment on cell-mediated immune *response* in carcinoma of the breast Acta radiol Ther Phys Biol 13 (1974), 83

ILBERY P L T, RICKENSON A B and THRUM C E Blood lymphocyte replicating ability as a measurement of radiation dosage Brit J Radiol 44 (1971), 834

JONDAL M, HOLM G and WIGZELL H Surface markers on human T and B lymphocytes J exp Med 136 (1972), 207

LAY W H and NUSSENZWEIG V Receptors for complement on leukocytes J exp Med 128 (1968), 991

on peripheral blood lymphocytes in patients with carcinoma of the breast Cancer 29 (1972) 349

MEYER K K and SAYRE P Radiation induced lymphocyte immune deficiency Arch Surg 101 (1970), 114

MICHLMAYR G and HUBER H Receptor sites for complement on certain human peripheral blood lymphocytes J Immunol 105 (1970), 670

MILLARD R E Effect of previous irradiation on the transformation of blood lymphocytes J clin Pathol 18 (1965) 783

NILSSON B S, SULTZER B M and BULLOCK W W Purified protein derivate of tuberculin induces immunoglobulin production in normal mouse spleen cells J exp Med 137 (1973), 127

PHILLIPS B and ROITT I M Evidence for transformation of human B lymphocytes by PHA Nature New Biology 241 (1973) 254

ROITT I M, GREAVES M F, TORRIGLANTI G, BROSTOFF J and PLAYFAIR J H The cellular basis of immunological responses Lancet 2 (1969), 367

SELZER P M and NILSSON B S PPD tuberculin acts as a mitogen for bone derived lymphocytes only Nature New Biology 240 (1972), 198

SEN I and BORELLA L Expression of cell surface markers on T and B lymphocytes after long

for mammary carcinoma Lancet 24 (1972), 1352

THOMAS J M COY P, LEWIS H S and YUEN A Effect of therapeutic irradiation on lympho cyte transformation in lung cancer Cancer 27 (1971), 1046

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TREATMENT OF PINEALOMAS BY STEREOTAXIC RADIATION SURGERY

ERIK-OLOF BACKLUND, TIIT RAHN and BERT SARBY

Tumours in the pineal region have always posed therapeutic problems when radical excision has been attempted. Experiences from various types of surgery in 140 cases (1926—1962) were published by OLIVECRONA (1967), with an overall mortality as high as 50 per cent During the last ten years, more conservative modes of treatment have been used, including shunting procedures and radiation therapy.

At this department, stereotaxic radiation surgery has been applied to five cases of tumour in the pineal region with rewarding preliminary results in all cases, as a matter of course without complications or mortality Three of these had comparatively small tumours, not causing much deformity of the CSF pathways. Furthermore, in these three cases, stereotaxic needle biopsy before the irradiation had given only scanty material which was not conclusive for diagnosis The remaining two cases were selected for presentation, as in each of these, a large tumour was present occluding the CSF passage, the stereotaxic biopsy was conclusive and the cytologic appearance that of a pineocytoma, and a *thorough follow-up including pneumography was performed*, giving evidence of a considerable shrinkage of the lesion

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Fig 1 Case 1 Preoperative encephalography Deformation of the posterior part of the third ventricle by a rounded pinealoma Curves of the dose distribution used in this case are superimposed indicating 80 60 40 and 20 per cent isodose levels in relation to the dose at the four target points two of which are indicated by crosses in this figure

Method The method of treatment has been fully described previously (LEKSELL 1971, BACKLUND et coll 1972) and will only be briefly mentioned here The procedure is performed by cross firing of the tumour by narrow beams from 179 small ^{60}Co sources, distributed over a spherical sector The beams are radially directed towards the centre of this sector, which is aligned with the stereotaxically predetermined tumour target point (or points) during the irradiation procedure The apparatus (manufactured by AB Motala Verkstad, Motala, Sweden) was primarily developed for functional neurosurgery Because of this, the system of apertures for the individual beams and the spatial distribution of the ^{60}Co sources were designed to give disc-shaped dose distributions For tumour treatment, such a dose distribution may be too small and not of optimal shape To overcome this and in order to align the irradiated volume to the shape and size of the tumour, a number of targets in a predetermined spatial pattern are used (Fig 1) As may be seen from the figure, the dose edge gradient is very steep, thus permitting large doses to be given to the target area while negligible doses are delivered to the surrounding tissues The irradiation is given in single doses and the dose rate at present permits the delivery of 1 000 rad to the target in the centre of a human head in about 10 minutes

Case reports

Case 1 A 1? ... of heada... protrusio... ...y a deformation of the posterior

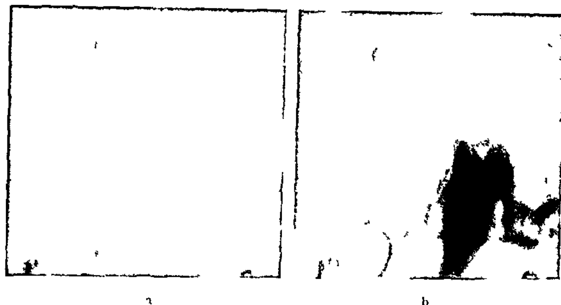


Fig. 2. Case 1. Encephalography (a) before and (b) one year after radiation surgery. In (b) the tumour has diminished in size almost to that of a normal pineal gland and the aqueduct appears normal.

part of the third ventricle, indicating a rounded pinealoma 2 cm in size was demonstrated (Figs. 1, 2 a). The aqueduct was displaced and partially obstructed, and marked ventricular dilatation was present (Fig. 3 a). No tumour vessels were seen at vertebral angiography. A stereotaxic aspiration biopsy of the tumour was performed and the cytologic appearance indicated a pineocytoma. The tumour was treated by stereotaxic radiation surgery. Four targets were chosen to given a dose distribution as optimal as possible (Fig. 1). A dose of 5 000 rad was delivered to each target, hardly any part of the tumour receiving less than 500 rad. The operation was performed under local anaesthesia. Postoperatively the papilloedema disappeared rapidly and two weeks later no protrusion could be seen. Simultaneously the symptoms and signs of raised intracranial pressure were relieved. At encephalography one year later the appearance of the tumour region was almost normal (Fig. 2 b) and the lateral ventricles of ordinary size and shape (Fig. 3 b). On re-examination three years after the treatment the patient was perfectly healthy and the ocular fundi were normal. Another encephalography revealed no obvious further change, although the pineal lesion was possibly still smaller.

Case 2. A 21 year old man was admitted in February 1972 with a history of delayed puberty and diplopia, upward gaze disturbances, headache and polyuria for six months. A previous encephalography had revealed a hydrocephalus apparently caused by a rounded expanding lesion about 2.5 cm in diameter partially occluding the aqueduct and causing dislocation of the third ventricle (Fig. 4 a). On admission panhypopituitarism was also established and the patient was subjected to full substitution. By stereotaxic biopsy the lesion was found to be a pineocytoma. In April 1972 the tumour was treated by stereotaxic radiation surgery. Four targets were chosen, with a distribution similar to that used in Case 1. The radiation dose to each target was 5 000 rad. One week after the treatment papill-

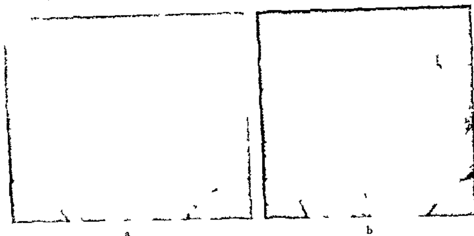


Fig 3 Case 1 The ventricular system before (a) and one year after the treatment (b) The width of the lateral ventricles is less in (b), whereas the third ventricle is still somewhat widened

oedema and symptoms of increasing intracranial pressure appeared This was assumed to be due to occlusion of the aqueduct caused by oedema of the tumour, induced by irradiation A ventriculo-atrial shunt ad modum Pudenz was inserted The ventricular pressure was found to be considerably raised After the shunt operation a rapid improvement was recorded One month later the shunt was found to be working inefficiently, nevertheless the patient was still improving Four months after the radiation surgery the papilloedema and the diplopia had disappeared but a slight weakness of upward gaze remained At lumbar encephalography only the basal cisterns and the fourth ventricle could be filled, ventriculography revealed that the tumour had disappeared but no passage of air through the aqueduct could be demonstrated This was considered to be due to a localized angulation of the aqueduct *in the vicinity* of the irradiated area possibly resulting from the tumour shrinkage A complete obstruction of the aqueduct was however not considered likely because of clinical evidence of adequate passage of CSF, as the papilloedema and subjective symptoms of intracranial pressure subsided in spite of the increasing malfunction of the shunt The patient was subsequently transferred in very good condition for further evaluation of his hormonal state A recent neurologic examination failed to reveal any defects apart from a slight weakness of upward gaze (March 1973)

Discussion

In spite of modern techniques, the surgical extirpation of pinealomas is still a problem and even in recent publications high mortality rates are reported SUZUKI & IWABUCHI (1965), in their presentation of 19 cases, gave an account of a 37 per cent operative mortality In 13 cases, a complete removal could be performed, but in the remainder (32 per cent), the removal was less than total



Fig 4 Case 2 a) Encephalography before treatment The tumour is indenting the third ventricle and causing considerable antero inferior displacement of the aqueduct and fourth ventricle b) Composite drawing from encephalography and ventriculography four months after radiation surgery The shape of the third ventricle is almost normal The fourth ventricle and the aqueduct were outlined by lumbar air injection

to a varying degree Of the lesions that were removed completely, four were cystic In the majority of the successful cases, shunt procedures and postoperative irradiation were also performed

In a series of 14 cases treated after 1950 with modern surgical techniques, POPPEN & MARINO (1968) reported only two fatal cases However, complete removal was attempted in three cases only and, furthermore, the two fatal cases belonged to this group These authors conclude 'Even with the several techniques now available, the postoperative mortality rate is still high , and a conservative attitude was advocated

Recently, 6 cases of lesions in the pineal region were reported by STEIN (1971), including one cystic astrocytoma, one epidermoid and one obscure case with arachnoidal cysts in the quadrigeminal region Subtotal removal was performed in two cases, another two were submitted to biopsy and in the remaining two only cyst aspiration and exploration plus a shunting procedure were performed, respectively No immediate postoperative mortality was reported, but the astrocytoma patient died one year after surgery

A further six cases were reported by JAMIESON (1971), obviously constituting selected surviving cases from a larger unpublished series The histologic grouping of these six cases was atypical teratoma (3), pineocytoma (1), undifferentiated cyst (1), and normal pineal gland? (1) A fairly radical removal was achieved but in three cases gaze disturbances and other preoperative signs remained

unchanged after operation In one case, it was necessary to make an occipital lobe resection, which caused a permanent hemianopia

In general, those neurosurgeons who have made use of more conservative measures in pinealoma treatment report better clinical results

CUMMINS et coll (1960) assessed the value of radiation therapy in 37 cases of tumour of the third ventricle (22) and pinealomas (15) The operative mortality reported after direct surgical approach for excision was 34 per cent The patients who were exposed to less radical surgery and were irradiated postoperatively had a 61 per cent five year survival rate The authors are definite in their conclusion that the preferred method is radiation alone or combined with shunting, if obstructive hydrocephalus is present In a paper by SMITH (1961), presenting a large series including cases previously reported by RAND & LEMMEN (1953), a similar conclusion was drawn

It is interesting to note the change of opinion of SUZUKI in his presentation of a second pinealoma series (SUZUKI & HORI 1969), in which he advocated shunting and radiation therapy The authors quote several Japanese colleagues with similar experiences One fatal case in this latter series however indicates the potential risks with high voltage irradiation to targets close to the brain stem Fortunately, disastrous courses after radiation therapy in similar cases are infrequent, but illustrate the main drawbacks of this type of treatment (GHATAK & WHITE 1969, HOLDORFF & SCHIFFTER 1971) Such disadvantages can be avoided and the risks reduced to a minimum by stereotaxic radiation surgery, whereby the radiation is delivered almost exclusively to the tumour, making possible the selective irradiation of neoplasms in the pineal region, and at ectopic sites separately

In order to utilize maximally the intrinsic precision of this type of treatment, a precise radiologic mapping of the tumour is a prerequisite Encephalography and vertebral angiography make such an accurate mapping possible and in many cases also permit a determination of the character of the tumour (LOFGREN 1958 GREITZ 1972)

It is often stated that any course of radiation therapy should be based upon histologic verification of the lesion to be irradiated In radiation surgery on the other hand this principle is less critical as the purpose is complete necrosis of the irradiated area Nevertheless stereotaxic biopsy was performed in the two cases presented, for two main reasons (1) Cystic lesions should not be treated with closed radiation surgery, but may be suitable objects for intracystic isotope treatment (BACKLUND et coll) (2) Histologic classification certainly influences the determination of the dose level, as radiation doses higher than the minimum dose necessary for necrosis are pointless

Fractionated radiation doses of 3 500 to 6 200 rad (SUZUKI & HORI), 4 000 to 5 000 rad (CUMMINS et coll.) and 3 500 to 4 200 rad (ENNUYER et coll. 1956) delivered with a conventional technique have proved to be fully effective in preventing continued growth of pineal tumors. As the biologic effect produced by a single dose is greater than that produced by the same dose fractionated, single doses of 5 000 rad have been considered sufficient to cause obliteration of the two pinealomas presented. A considerable shrinkage of the tumours was evident although complete disappearance of the lesions could not be demonstrated. The radiologic findings after the treatment may however be explained by the fact that tumour remnants after irradiation may consist of a non-vital mass of debris (SUZUKI & HORI).

With the introduction of stereotaxic radiation surgery, new possibilities are offered for treating other benign and circumscribed but relatively inaccessible lesions of the brain, such as craniopharyngiomas and other pituitary tumours as well as acoustic neurinomas.

This type of therapy has been used in a series of such cases since 1968 with encouraging results. In craniopharyngiomas and pituitary adenomas not only a permanent obliteration and shrinkage of the tumour has been demonstrated but also, in many cases, improvement of the endocrine state of the patients (BACKLUND 1973, 1974). In the field of acoustic neurinoma treatment, it has been possible, in a small series, to irradiate selectively tumour remnants in the orifice of the internal meatus after conventional surgery. The experiences hitherto indicate that radiation doses sufficiently great to cause shrinkage of the tumour remnants can be delivered without simultaneously being noxious to the facial nerve. This is supported by the general conception in radiation biology that axons are very resistant to irradiation (cf. RUBIN & CASARETT 1968). For small acoustic tumours, radiation surgery has been used as primary treatment and one case has been reported (LEKSELL 1971).

With the method of treatment presented, it has been possible to obtain striking therapeutic effects both as regards the tumours and the clinical condition of the patients. The clinical course in these two patients furthermore was very smooth, contrasting markedly with the often very dramatic postoperative course after radical surgery; stereotaxic radiation surgery is therefore considered the method of choice in cases of pinealoma.

SUMMARY

Two cases of pineocytoma, treated by stereotaxically directed gamma radiation from 179 ^{60}Co sources are presented. Before treatment the tumours were classified by stereotaxic fine needle biopsy. In both cases signs and symptoms of raised intracranial pressure

disappeared soon after the irradiation Encephalography revealed almost complete disappearance of both lesions The advantages of this type of pinealoma treatment as well as the general principles for stereotaxic radiation surgery in cases of small, benign, inaccessible intracranial tumours are discussed

ZUSAMMENFASSUNG

Zwei Falle mit einem Pineocytom, die mit stereotaktisch gerichteter Gamma Bestrahlung durch 179 ^{60}Co Strahlenquellen behandelt worden waren, werden vorgestellt Vor der Behandlung waren die Tumoren mittels stereotaktischer Feinnadelbiopsie klassifiziert worden In beiden Fallen verschwanden die Zeichen und Symptome eines gesteigerten intrakranialen Drucks rasch nach der Bestrahlung Die Encephalographie ergab ein beinahe vollstandiges Verschwinden beider Lasionen Der Fall der Behandlung eines Pinealoms sowie die generellen Prinzipien der stereotaktischen Strahlenchirurgie bei Fallen mit kleinen, benignen, nicht erreichbaren intrakranialen Tumoren werden diskutiert

RÉSUMÉ

Presentation de deux cas de pineocytome, traites par la radiation gamma de 179 sources de ^{60}Co Avant le traitement les tumeurs ont ete classées par biopsie stereotaxique avec une aiguille fine Dans les deux cas les signes physiques et les signes fonctionnels d hypertension intracrânienne ont disparu peu de temps apres l irradiation L'encéphalographie a montre une disparition presque complete des deux lesions Les auteurs examinent le cas du traitement des pinealomes ainsi que les principes generaux de la radiochirurgie stéréotaxique dans les cas de petites tumeurs intracrâniennes benignes inaccessibles

REFERENCES

BACKLUND E O, JOHANSSON L and SARBY B Studies on craniopharyngiomas II Treatment by stereotaxis and radiosurgery Acta chir scand 138 (1972), 749

BACKLUND F O Studies on craniopharyngiomas IV Stereotaxic treatment with radiosurgery Acta chir scand 139 (1973), 344

— Indications for and results of treatment with stereotaxic radiosurgery *In* D[illegible]

[illegible]

ENNUYER A, GUIOT G, CHECUILLAUME J et GUENOT J. À propos de la radiothérapie des tumeurs de la région épiphysaire Sem Hop Paris 32 (1956), 1955

GHATAK N R and WHITE B E Delayed radiation necrosis of the hypothalamus Arch Neurol 21 (1969), 425

GREITZ T Tumours of the quadrigeminal plate and adjacent structures Acta radiol Diagnosis 12 (1972), 513

HOLDORFF B und SCHIFFTER R Strahlenspatnekrosen des Hirnstammes einschliesslich Hypothalamus nach Bestrahlung mit ultraharten Röntgenstrahlen und schnellen Elektronen Acta neurochir 25 (1971), 37

JAMIESON K G Excision of pineal tumor J Neurosurg 35 (1971), 550
LEKSELL L Stereotaxis and Radiosurgery Charles C Thomas Springfield Ill 1971
— A note on the treatment of acoustic tumours Acta chir scand 137 (1971), 763
LOFGREN I O Vertebral angiography in the diagnosis of tumours in the pineal region Acta radiol 50 (1958), 108
OLIVECRONA H The surgical treatment of intracranial tumours *In* Handbuch der Neurochirurgie, Band IV/4 Edited by H Olivecrona and W Tonnis Springer Verlag Berlin 1967
POPPEN J L and MARINO R Pinealomas and tumors of the posterior portion of the third ventricle J Neurosurg 28 (1968), 357
RAND R W and LEMMEN L J Tumors of the posterior portion of the third ventricle J Neurosurg 10 (1953), 1
RUBIN P and CASARETT G W Clinical radiation pathology W B Saunders Philadelphia 1968
SMITH R A Pineal tumors Univ Mich med Bull 27 (1961) 33
STEIN B M The infratentorial supracerebellar approach to pineal lesions J Neurosurg 35 (1971) 197
SUZUKI J and IWABUCHI T Surgical removal of pineal tumors (Pinealomas and teratomas) J Neurosurg 23 (1965), 565
— and HORI S Evaluation of radiotherapy of tumors in the pineal region by ventriculographic studies with iodized oil J Neurosurg 30 (1969), 595

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QUANTITATIVE LONG-TERM DETERMINATIONS OF THE ALVEOLAR BONE MINERAL MASS IN MAN BY ^{125}I ABSORPTIOMETRY

I Accuracy and precision of the method

CARL O HENRIKSON and JAN BERGSTROM

In a roentgenogram of the alveolar bone its structure is well demonstrated It is, however, difficult to evaluate small changes in the mineral content of the alveolar bone by means of conventional radiographic techniques OMNELL (1957) developed a *roentgenographic photometric* method for measuring changes in the mineral content of alveolar bone The recording of small mass changes by roentgenography was analysed by HOLLENDER & LYSELL (1972) and LYSELL (1973), who found that monoenergetic radiation without evident scatter radiation increased precision and accuracy

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JAMIESON K G Excision of pineal tumor J Neurosurg 35 (1971) 550

LEKSELL L Stereotaxis and Radiosurgery Charles C Thomas Springfield Ill 1971

— A note on the treatment of acoustic tumours Acta chir scand 137 (1971), 763

LOFGREN F O Vertebral angiography in the diagnosis of tumours in the pineal region Acta radiol 50 (1958), 108

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POPPEN J L and MARINO R Pinealomas and tumors of the posterior portion of the third ventricle J Neurosurg 28 (1968), 357

RAND R W and LEMMEN L J Tumors of the posterior portion of the third ventricle J Neurosurg 10 (1953), 1

RUBIN P and CASARETT G W Clinical radiation pathology W B Saunders Philadelphia 1968

SMITH R A Pineal tumors Univ Mich med Bull 27 (1961), 33

STEIN B M The infratentorial supracerebellar approach to pineal lesions J Neurosurg 35 (1971), 197

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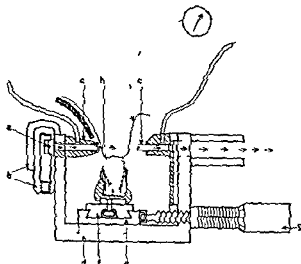


Fig 1 The ^{125}I apparatus a) radiation source b) radiation shielding c) collimators in form of steel cylinders d) base plate e) and f) movable slides g) micrometer screw (From HENRIKSON & JULIN 1971)

1.0 mm. The source, with the collimators, is movable with micrometer screws in mesio-distal as well as in labio lingual direction (Fig 1)

The radiation is recorded to nearly 100 per cent by a NaI (Tl) scintillation crystal, pulse height analyser and scaler (Nukab 9 700). The scintillation crystal is 25 mm in diameter and 2.5 mm thick. The thin scintillation crystal without any special shielding but with discrimination of the background radiation outside the region of interest provides a background of less than 50 counts per minute. However, the counting rates were at least two hundred times higher than this background. Apart from that the background was not subject to any fluctuations other than statistical ones. The background radiation has not been taken into consideration in the calculation of the bone mineral mass.

When measuring the thickness of the alveolar process the collimators serve as registrators. The collimators are electrically insulated from the rest of the apparatus and connected one at a time, by a cable to a resistance meter. The other cable of the instrument is connected to the subject. When contact is attained between the gingiva and the collimator a current passes through the subject, causing a deflection of the resistance instrument. The position of the collimator when in contact is read on the micrometer scale. Knowing the distance between the two collimator cylinders, the thickness of the alveolar process can be calculated.

During the last decade great efforts have been made to develop methods for determining bone mineral content using roentgen spectrometry (JACOBSON 1964), or radiation of isotopes (CAMERON & SORENSEN 1963, SCHMELING 1972). Most frequently ^{125}I is used, introduced for odontologic roentgenography by BERONIUS et coll. (1962). An isotope method for measurement of the mineral content of alveolar bone was described by HENRIKSON (1967) and further developed by HENRIKSON & JULIN (1971).

The purpose of the present investigation was to determine the errors of the method and the variations of alveolar bone mass in subjects with healthy periodontia during different lengths of observation periods.

Material. Eleven students, 4 females and 7 males aged 23 to 28 years, at the Dental School, Stockholm, volunteered. They presented good periodontal health without roentgenologic evidence of any bone destruction marginally, dental plaque, if any, was removed. The region chosen for the bone mineral measurements was invariably the alveolar bone between a lateral incisor and a canine of the upper jaw.

Method

The radiation source, with a diameter of 0.5 mm, is composed of ^{125}I plated in the form of silver iodide on the flat end surface of a silver wire. The radiation from ^{125}I has the main energy 27.4 keV and small contributions of 31.1 and 35.5 keV radiation. Fluorescence radiation from silver with energies of 22.2 and 25.0 keV is also apparent from the source. In order to reduce all radiation energies in relation to the 27.4 keV radiation, a 0.1 mm thick tin filter was used.

The spectrum of the radiation from the Ag^{125}I source was obtained with a planar high-purity silicon surface barrier detector with a 0.25 mm thick window of beryllium. The thickness of the detector was 5 mm and the diameter 18 mm. When calculating the relative intensity of the individual components of the radiation, the efficiency of the silicon detector at these energies has been taken into consideration (FORBERG 1973).

Apparatus. The principles for the ^{125}I apparatus have been described by HENRIKSON (1967). Modifications of the apparatus for measuring changes in radiation transmission through a certain part of the alveolar process, as well as for measuring the thickness of the alveolar process, were made by HENRIKSON & JULIN (1971). The ^{125}I radiation source is mounted in a cylindrical brass shielding and the radiation beam is collimated with the aid of two steel cylinders, one on each side of the subject. The inner diameter of the collimators is

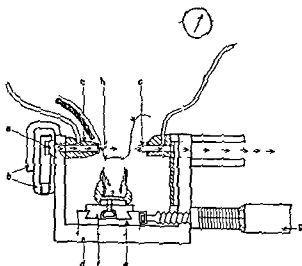


Fig 1 The ^{125}I apparatus a) radiation source b) radiation shielding c) collimators in form of steel cylinders d) base plate e) and f) movable slides g) micrometer screw (From HENRIKSON & JULIN 1971)

1 0 mm The source, with the collimators, is movable with micrometer screws in mesio-distal as well as in labio-lingual direction (Fig 1)

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When measuring the thickness of the alveolar process the collimators serve as registrators The collimators are electrically insulated from the rest of the apparatus and connected one at a time, by a cable to a resistance meter The other cable of the instrument is connected to the subject When contact is attained between the gingiva and the collimator a current passes through the subject, causing a deflection of the resistance instrument The position of the collimator when in contact is read on the micrometer scale Knowing the distance between the two collimator cylinders, the thickness of the alveolar process can be calculated



Fig. 2. The ^{125}I apparatus mounted on cap splint. The arrow indicates the measurement area.

Recording procedure. In order to obtain a reproducible position of the radiation beam in relation to the part of the alveolar bone to be examined the apparatus with source and collimators was rigidly screwed to a cap splint (Fig. 2). The cap splint was made of silver and covered the crowns of the teeth and part of the palate.

Before measurements on a subject the orientation of the apparatus into a desired position was performed on a plaster model of the jaw; at the same time information concerning the anatomic details was obtained from films of the region. The height position was thereby fixed.

The final selection of the part of the alveolar process to be measured was made by a scanning procedure on the subject. The source was moved in a lateral direction approximately parallel to the alveolar process and teeth. The amount of transmitted radiation reflects the position of the beam in relation to the teeth and interdental septum (Fig. 3).

From this scanning procedure two positions, P_1 and P_2, with the highest transmission values were chosen as measurement points. The distance between such interdental points was 0.1 to 0.5 mm in this material.

At each observation the transmission was measured at both positions, P_1 and P_2. The procedure was then repeated after readjustment of the apparatus to the two positions. Thus the transmission value of a certain observation is based on two plus two single assessments, in the sequel denoted as replicate determinations. The counting time varied from 0.5 to 2.0 min and the number of counts, representing transmitted radiation, varied from 100 000 to 10 000 for each measurement of each point.

The thickness of the alveolar process at the region in question was determined at one position only, $\frac{(P_1 + P_2)}{2}$. The mean of two or three micrometer readings

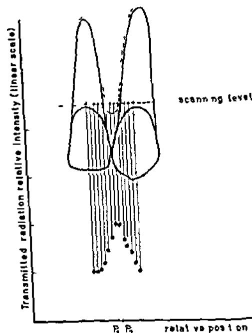


Fig 3 Drawing of the scanning procedure on the subject. The relative intensity of the transmitted radiation I_1/I_0 is recorded at equidistant intervals in the mesiodistal direction

palatally as well as labially was used when calculating the value of the total thickness

Calculation of bone mineral mass In passing through matter, monoenergetic radiation is transmitted according to the formula

$$\frac{I_1}{I_0} = e^{-\mu l} \qquad (1)$$

where I_1 = intensity of transmitted radiation

I_o = intensity of incident radiation

μ = linear attenuation coefficient (cm^{-1})

l = thickness of absorbing layer (cm)

The inorganic component of bone consists mainly of hydroxyapatite and to a less extent of carbonate apatite The inorganic components of the bone mineral may be approximated to hydroxyapatite for the energies used (OMNELL 1957) HENRIKSON & LINDEN (1974), using the same apparatus and a similar radiation source, found a linear attenuation coefficient of 9 4 ± 0 15 cm^{-1} (mean ± SD) for hydroxyapatite, determined from 200 μm thick enamel slices

The soft tissue of the alveolar process consists mainly of water, protein and fat and the linear attenuation coefficient was 0 45 cm^{-1} as determined from gingival biopsies (HENRIKSON & JULIN 1971)

It thus follows that the fraction of hydroxyapatite in the alveolar process may be calculated from formula (1)

$$\frac{I_1}{I_0} = e^{-[9.4x + 0.45(1-x)]} \quad (2)$$

where x = length of hydroxyapatite (cm)
l = total thickness (cm)

Knowing the density of hydroxyapatite it is possible to express the mass of hydroxyapatite in terms of g cm" or mg mm A density of 3 1 g cm^{-3} is used for hydroxyapatite in this work (OMNELL 1957, ANGMAR et coll 1963 HODGMAN 1963)

Sources of errors The components of the total error of the method are besides geometric instability, errors of the counting procedure and of the constants used for the calculation of the bone mineral mass The first component the geometric instability, is related to changes in the relationships between (a) the source and the collimators (b) the base of the source holder and the slide (c) the slide and the cap splint, (d) the cap splint and the teeth and (e) the teeth and the alveolar bone

The errors under (a)—(c) were not significant compared to the standard error (30 8 μm) inherent in the stereophotogrammetric method applied for their determination (HENRIKSON 1967) In relatively long periods of observation the geometric instability may be higher than for short periods due to the factors (d) and (e) Furthermore it cannot be excluded that longer periods of observation may involve biologic variations of the mineral mass of the certain part of the bone These variations may be caused by bone metabolism as well as by variations of external factors e g oral hygiene The geometric instability the errors of the counting procedure and the constants used for the calculation of the bone mineral mass will be dealt with below

Experimental design The present investigation was divided into two parts First a determination was made of the precision of the method by means of replicate determinations within observation periods of *different length* as well as of the intraindividual variations for the different period and secondly a *theoretical analysis* was made of the magnitude of the errors originating from the counting procedure and from the constants used for the calculation of the bone mineral mass

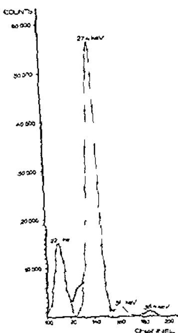


Fig 4 Spectral distribution of the radiation from the Ag^{17} I source obtained by a planar high purity silicon surface barrier detector

The measurements were performed in two sequences on 11 *subjects* I Two observations within 10 days at at least one day's interval *Each* observation consisted of two replicate determinations II *One observation* of two replicate determinations 120 to 180 days *after sequence I* in the same region During the first twenty days after sequence I *there* was a total absence of toothbrushing, followed by 100 *to* 160 days of good oral hygiene (BERGSTROM &

Th

tion

by

$$s_d = \sqrt{\frac{\sum d_i^2}{2n}} \qquad (3)$$

where d = difference between two replicate determinations of each observation and

n = number of differences

The accuracy of a certain parameter can be expressed by discrepancies (e_i)

Table 1

Relative intensities of the five energies from the $Ag^{125}I$ radiation source (filtration 0 1 mm tin) according to Fig 4 The figures have been corrected for detector efficiency

Type	Energy	Per cent of total
Kα rays of silver	22 1	15 6
Kβ rays of silver	25 0	4 4
Kα rays of tellurium	27 4	75 3
Kβ rays of tellurium	31 1	3 2
Gamma ray	35 4	1 5

Table 2

Calculated values of effective linear attenuation coefficient of hydroxyapatite for different thicknesses of bone mineral component at total thicknesses 1 0 cm and 1 5 cm The absorbing layer is supposed to consist of hydroxyapatite and water

Thickness of mineral component in cm	Effective linear attenuation coefficient (cm^{-1}), total thickness of absorbing layer	
	1 0 cm	1 5 cm
0 01	9 20	9 10
0 02	9 10	8 90
0 05	9 00	8 80
0 10	8 85	8 75
0 15	8 75	8 65
0 20	8 65	8 55

between measured values and given (or true) values (HALLERT 1967) according to

$$s_m = \sqrt{\frac{\sum e_i^2}{n}} \qquad (4)$$

The analyses of variance were performed according to standard procedures (MENDENHALL 1968)

Results

Accuracy

In calculating the values of bone mineral mass three parameters are used which may deviate from true values in a systematic way These three parameters

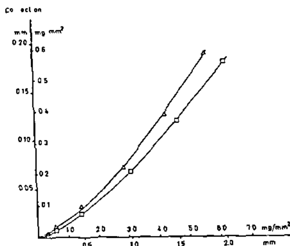


Fig 5 The correction for the 'hardening' effect calculated at two total thicknesses are plotted versus the values of the apparent mineral content (hydroxyapatite) calculated from a constant linear attenuation coefficient ($\mu = 9.4$)
△ Total thickness 1.50 cm
□ Total thickness 1.00 cm

are the linear attenuation coefficients used for bone mineral and soft tissue respectively, the thickness value and the density value used for bone mineral

Linear attenuation coefficients The calculation of the bone mineral mass is based on the linear attenuation coefficients 9.4 cm^{-1} and 0.45 cm^{-1} for the hydroxyapatite and soft tissue respectively These linear attenuation coefficients were, however, determined experimentally with relatively thin absorbing layers (HENRIKSON & LINDEN 1974) As the radiation from the source (Ag ^{125}I) is not strictly monoenergetic, a generally shorter wave length of the radiation may be expected ('hardening effect') This effect becomes more accentuated the thicker the absorbing layer

The spectral distribution of the radiation used (Fig 4) indicates that the fluorescence radiation from silver with energies 22.1 and 25.0 keV, the K-radiations from tellurium at 31.1 keV and the gamma radiation from ^{125}I, at 35.4 keV, must be considered (Table 1) The attenuation of a discrete spectrum of radiation can be generally described by the equation

$$\frac{I_1}{I_0} = \sum f_j \, e^{-\Sigma \mu_{k,j} \, l_k} \qquad (5)$$

where f_j = the fraction of radiation with j^{th} energy
$\mu_{k,j}$ = the linear attenuation coefficient of the k^{th} absorber at the energy of the j^{th} energy component (cm^{-1})
l_k = the thickness of the k^{th} absorber (cm)

Using formula (5) it is possible to calculate an effective linear attenuation coef-

Table 1

Relative intensities of the five energies from the $Ag^{125}I$ radiation source (filtration 0.1 mm tin) according to Fig. 4. The figures have been corrected for detector efficiency

Type	Energy	Per cent of total
$K\alpha$ rays of silver	22.1	15.6
$K\beta$ rays of silver	25.0	4.4
$K\alpha$ rays of tellurium	27.4	75.3
$K\beta$ rays of tellurium	31.1	3.2
Gamma ray	35.4	1.5

Table 2

Calculated values of effective linear attenuation coefficient of hydroxyapatite for different thicknesses of bone mineral component at total thicknesses 1.0 cm and 1.5 cm. The absorbing layer is supposed to consist of hydroxyapatite and water

Thickness of mineral component in cm	Effective linear attenuation coefficient (cm^{-1}), total thickness of absorbing layer	
	1.0 cm	1.5 cm
0.01	9.20	9.10
0.02	9.10	8.90
0.05	9.00	8.80
0.10	8.85	8.75
0.15	8.75	8.65
0.20	8.65	8.55

between measured values and given (or true) values (HALLERT 1967) according to

$$s_m = \sqrt{\frac{\sum e_i^2}{n}} \quad (4)$$

The analyses of variance were performed according to standard procedures (MENDENHALL 1968).

Results

Accuracy

In calculating the values of bone mineral mass three parameters are used which may deviate from true values in a systematic way. These three parameters

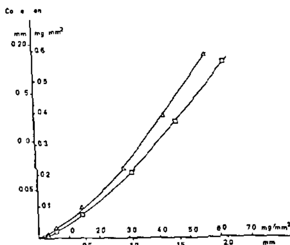


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l_k = the th[illegible]
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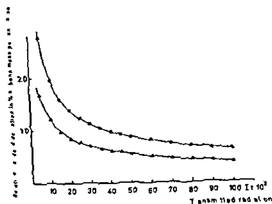


Fig 6 Relative standard deviation from counting statistics (relative counting error), given as per cent of bone mineral mass versus number of accumulated transmitted counts ($I_t t_t$) for transmissions (I_t/I_o) 0 25 and 0 35 at a total tissue thickness of 1 030 cm

relative standard deviations $\frac{s_o}{I_o}$ and $\frac{s_x}{I_x}$ of the incident and transmitted radiations according to

$$\left(\frac{s_T}{T_x}\right)^2 = \left(\frac{s_o}{I_o}\right)^2 + \left(\frac{s_x}{I_x}\right)^2, \text{ hence}$$

$$s_T = T_x \sqrt{\frac{1}{I_o t_o} + \frac{1}{I_x t_x}} \tag{7}$$

The influence of this relative counting error upon the value of bone mineral mass was estimated for the present sample of individuals Two transmission values 0 25 and 0 35 representing the upper and lower limits, and a total thickness of absorbing layer of 1 030 cm (mean of the sample) were used

In Fig 6 the magnitude of the relative counting error, expressed in per cent of the value of bone mineral mass per unit area is plotted versus the number of transmitted quanta

It is evident that the relative counting error becomes more marked as the number of transmitted quanta decreases, its influence is more accentuated the higher the transmission (i e the thinner the bone) As the intensity of the isotope decreases with the decay of the source, the influence of this error can be reduced by an extension of the counting time For transmission levels corresponding to those presented here and representing measurements of alveolar bone, a number of transmitted counts of at least 15 000 to 35 000 will keep this error below 1 per cent of the resulting value of mineral mass (Fig 6)

The thickness measurement The precision of the thickness measurement procedure was estimated from repeated readings labially as well as palatally at

ficient for different thicknesses of the absorbing layer consisting of two components (hydroxyapatite and soft tissue)

For an absorbing layer of 0 200 mm hydroxyapatite and 0 028 mm water the calculated effective linear attenuation coefficient was 9 3 cm^{-1}, and this is in agreement with the value of 9 4 ± 0 15 cm^{-1} experimentally arrived at by HENRIKSON & LINDÉN (1974), who used enamel slices of approximately the same thickness The attenuation coefficients for the two components for the five energies have been derived from OMNELL (1957)

In order to increase the accuracy of the bone mineral mass value, the effective linear attenuation coefficients were calculated for different thicknesses of absorbing layers (Table 2) From the diagram in Fig 5 it is possible to make corrections for the values of bone mineral mass obtained with the linear attenuation coefficients of 9 4 cm^{-1} and 0 45 cm^{-1} for hydroxyapatite and water respectively

Thickness measurement Changes in thickness of the alveolar process may affect the value of bone mineral mass The influence of systematic errors due to use of the micrometer scale of the apparatus was tested by measuring objects of known lengths (Johansson's Gauge Blocks) The accuracy of this procedure was (s_M) 0 08 mm

The density of hydroxyapatite used is given above

Precision of the recording technique

The random errors affecting values of bone mineral mass are functions of errors in the determination of the transmission and of errors in the determination of the thickness

Counting statistics When measuring the intensity of radiation by means of an event counter, the contribution of count statistics to the uncertainty of the count rate I_1 is conveniently expressed by the standard deviation s Throughout this work the background is negligible in relation to the net counts Hence s is equal to the ratio between the square root of the accumulated count number m and the counting time t_1 (FRIEDLANDER & KENNEDY 1955)

$$s_1 = \frac{\sqrt{m_1}}{t_1} = \frac{\sqrt{I_1 t_1}}{t_1} = \sqrt{\frac{I_1}{t_1}} \quad (6)$$

For a transmission $T_x = \frac{I_x}{I_0}$ the relative standard deviation $\frac{s_T}{T_x}$ depends on the

Table 4

Analysis of variance of values of bone mineral mass ($mg\ mm^{-2}$) pertaining to sequences I + II The variation due to the interaction between subjects and observations is significant ($p < 0.001$) as well as between observations is significant ($p < 0.05$) The variation between subjects is not considered

Source of variation	D F	Sum of squares	Mean square	Expected mean square
Between subjects	10	27.30422	2.73042	
Between observations	2	0.00904	0.00452	$\sigma^2+2\sigma^2_{int}+20\sigma^2_{obs}$
Interaction	20	0.18184	0.00909	$\sigma^2+2\sigma^2_{int}$
Residual	33	0.02856	0.00087	σ^2

$F\frac{int}{res} = 10.448^{***}$ $F\frac{obs}{res} = 5.195^{*}$ $F\frac{int}{obs} = 2.011$

In order to further evaluate the components of variation connected with measurements in vivo, an analysis of variance (three components) was performed for sequence I and for sequences I + II (Tables 3 and 4 respectively) The methodologic component of variation (residuals) is small compared to the fluctuations between observations within subjects, i e the differences in mineral mass values between observations are greater than can be expected from repetition of the measurement procedure alone

This is also apparent on an extension of the time between observations as in sequence I + II (Table 4) Here the effect from interaction between subjects and observations represents a dominant part of the total variation and is significantly increased compared to sequence I ($F = 3.61$, $p < 0.05$) This is interpreted in the manner that the increase of variation tends to be characteristically related to the subject, some subjects tending to vary more (or less) than in general, while most subjects remain constant

Discussion

The radiation source, $Ag^{125}I$, is made by electrolytic deposition (BERONIUS et coll 1965, HENRIKSON 1967) The relative fraction of radiation from silver was 20 per cent (Table 1), which, in spite of the tin filtration, is larger than reported by HENRIKSON (1967) The somewhat different spectral distribution found in the present investigation is mainly a result of the higher resolution power of the semiconductor detector system now used The 'hardening' effect is

Table 3

Analysis of variance of mineral mass values (mg mm ²) pertaining to sequence I Significant variation is obtained between observations ($p < 0\ 05$) and for the interaction between subjects and observations ($p < 0\ 01$) The variation between subjects is not considered

Source of variation	D F	*Sum of squares*	*Mean square*	*Expected mean square*
Between subjects	10	17 61913	1 76191	
Between observations	1	0 00337	0 00337	$\sigma^2 + 2\sigma^2_{int} + 22\sigma^2_{obs}$
Interaction	10	0 02520	0 00252	$\sigma^2 + 2\sigma^2_{int}$
Residual	22	0 01461	0 00066	σ^2

$F \frac{int}{res} = 3\ 818^{**}$ $F \frac{obs}{res} = 5\ 106^{*}$ $F \frac{obs}{int} = 1\ 337$

each observation The standard deviation for a single thickness determination, as a mean throughout the observation period, was 0 09 mm

Precision of the method in vivo

The 'methodologic' component of the total variation during observation sequences is reflected in the deviation of the replicates from each other according to formula (3), i e the precision of the method in vivo The remainder of the total variation is thought to represent the 'biologic' variation The precision for sequences I and II was 0 028 and 0 035 mg mm ² respectively Expressed in per cent of the mean value of bone mass for these sequences (2 730 and 2 708 mg mm ²) the methodologic error amounted to 1 02 and 1 29 per cent, respectively

Intraindividual variation between observations was determined by treating the differences between observations according to formula (3) First intraindividual differences between the mean values of the two observations within sequence I were treated The standard deviation thus obtained for sequence I (short time differences) was 0 038 mg mm Then, in the same manner, differences were formed between the mean values of the first observation and the second observation, respectively, of sequence I and the mean values of the one observation of sequence II Now the standard deviation (long-time differences) was 0 070 mg mm and 0 083 mg mm ² respectively (or 2 5 to 3 per cent) No systematic tendency was discovered regarding these long-time differences

Statistical analysis (F-test) shows a significant difference between long time and short-time intraindividual variation ($F = 3\ 35$ and $4\ 47$ respectively, $p < 0\ 05$, $df = 10$)

keep part of this effect under control, series of replicated determinations in the subject are advocated

The technique described was designed for measurement of the bone mineral mass of the marginal part of the alveolar process There are some advantages in using this location for such measurements, the soft tissue cover is thin and the thickness of the part of the alveolar process in question is easily accessible for recording, furthermore the teeth serve as elements of fixation for the necessary reproducibility Finally, another advantage of this method is the extremely low surface dose to the subject (less than 16 mR per observation) in a very small irradiated volume (HENRIKSON 1967) The method is thought to be appropriate for quantitative evaluation of influences on the bone mineral mass from external local factors as well as from internal factors of a metabolic character

SUMMARY

The mineral mass of the alveolar interdental bone was measured in young adults with healthy periodontia using ^{125}I absorptiometry The precision was 1 per cent and intra individual variation over a period of 180 days was 2 to 3 per cent The ^{125}I radiation source is not strictly monoenergetic The magnitude of the shortening of the wave length for different thicknesses of the bone was investigated

ZUSAMMENFASSUNG

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ener [illegible] wurde die Grosse der Verkurzung der Wellenlange fur verschieden starke Knochen untersucht

RÉSUMÉ

La teneur minerale de l os alveolaire interdentaire a ete mesuree chez des adultes jeunes ayant un périodonte sain au moyen de l absorptiometrie de ^{125}I La precision a ete de 1 pour cent et la variation intra individuelles sur une periode de 180 jours a ete de 2 à 3 pour cent La source de rayonnement ^{125}I n est pas strictement monoenergetique Les auteurs ont etudie l amplitude du raccourcissement de longueur d onde pour differentes epaisseurs de l os

REFERENCES

ANGMAR B, CARLSTROM D and GLAS J E Studies on the ultrastructure of dental enamel IV The mineralisation of normal human enamel J ultrastruct Res 8 (1963), 12

BERGSTROM J and HENRIKSON C. O Quantitative long time determinations of the alveolar bone mineral mass in man by ^{125}I absorptiometry III Effect of experimental dental plaque formation To be published in Acta radiol Ther Phys Biol 14 (1975)

of such a magnitude that it has to be corrected for when high accuracy is needed

The *disturbing effect from silver radiation* is a consequence of the technique used for the production of the source For future work development of this technique using other deposition metals (e g copper) should be advantageous (BERONIUS et coll 1972) Another approach, the production of ^{125}I sources by ion exchange technique (CAMERON & SORENSON 1963), might also be developed for manufacturing satisfactory sources

The accuracy of the present method for alveolar bone mineral mass determination was not experimentally tested against known standards or standards regarded as being true However the technique of ^{125}I absorptiometry was found to be of high quality (4 to 7 %) by CAMERON et coll (1968) They reported a close correlation between bone mineral determinations and bone weight (r = 0 96 to 0 99) It seems reasonable to assume that the accuracy of the present method will be of the same order of magnitude

The precision of the method under in vivo conditions or the methodologic error was approximately 1 per cent as estimated by replication of the measurement procedure *It is to be noted that part of this error is statistical uncertainty* of the radiation recorded As the isotope decays with time, the uncertainty in the number of recorded radiations per unit of time will be a factor of importance *and must be taken into consideration Thus a number of accumulated counts* from transmitted radiation of about 15 000 to 35 000 (depending on the thickness of the bone to be measured) yields a 1 per cent error from the radiologic *recording procedure alone*

One way to compensate for the decreasing intensity is to accumulate the radiation during a longer period of time, i e to extend the counting time *However, there is a practical limit for convenient measurements in vivo* It was found that a recording time of more than two minutes cannot be recommended

Compared to the part of variation here denoted as the methodologic error, the intraindividual variation with time was larger and also increased on an extension of the time between observations It was also suggested that the magnitude of the intraindividual variation is specifically related to the subject to be measured

It is to be noted that during the first three weeks after sequence I the subjects were restrained from all forms of oral hygiene However, a possible effect of this insult to the mineral mass of the alveolar bone could not be detected at an observation made at 100 days after the start of the experiment (BERGSTROM & HENRIKSON 1974) Therefore it may be assumed that the experiment has not affected the mineral mass at the time for the 120 to 180 day observations

On the basis of the present sample of young individuals a total effect of errors of 2 to 3 per cent during long-term determinations is to be expected In order to

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POTENTIAL OF PROTON BEAMS FOR TOTAL NODAL IRRADIATION

JOHN O ARCHAMBEAU, GERALD W BENNETT and SONG TAO CHEN

Recent reviews of proton radiation therapy suggest an evident clinical role for the improved absorbed dose distribution available with proton beams (ARCHAMBEAU & BENNETT 1973, ARCHAMBEAU et coll 1974, KOEHLER & PRESTON 1972) (In the following the term absorbed dose is abbreviated to dose) The necessity of total nodal (or extended field) irradiation serves as a useful model to demonstrate the advantages to the patient of an improved dose distribution Treatment plans and dose distributions for treating a hypothetical case were derived for 4 MV roentgen rays and 200 MeV protons The normal tissue and tumor-containing volumes were specified, and the integral doses to these volumes were calculated The improved dose distribution should be manifested by an increased local control of irradiated cancer, as well as by a decreased patient morbidity (ARCHAMBEAU & BENNETT 1973, ARCHAMBEAU et coll 1974, KOEHLER & PRESTON 1972, STENSON 1971)

Approach A realistic tumor-containing volume 4 to 8 cm deep and 4 to 12 cm wide was arbitrarily designated along the central plane in the cervical, axillary, mediastinal, abdominal and pelvic regions of an Alderson Rando female phantom Hypothetical treatment plans using mantle and inverted Y fields were derived for 4 MV roentgen rays and 200 MeV protons (Fig 1)

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BERONIUS P, FORBERG S, HENRIKSON C O and SORFMARK R The use of Iodine 125 as an X ray source in roentgen diagnostics Int J appl Radiat 13 (1962) 253
— — — — Iodine 125 as a radiation source with special emphasis on its application in medical radiology II Ag I and Cu I as *point roentgen sources Acta radiol Ther Phys* Biol 3 (1965) 152
— JOHANSSON B and SORLMARK R Electrodeposition of point Cu 125 I roentgen sources AE 462 Studsvik 1972
CAMERON J R MAZESS R B and SORENSON J A Precision and accuracy of bone mineral determination by direct photon absorptiometry Invest Radiol 3 (1968) 141
— and SORENSON J A Measurement of bone mineral in vivo An improved method Science *142 (1963), 230*
FORBERG S Personal communication (1973)
FRIEDLANDER G and KENNEDY J W Nuclear and radiochemistry John Wiley & Sons Inc New York 1955
HALLERT B Elementär felteori för mätningar (In Swedish) Norstedt Stockholm 1967
HENRIKSON C O Iodine 125 as a radiation source for odontological roentgenology Acta radiol (1967) Suppl No 269
— and JULIN P Iodine 125 apparatus for measuring changes in X ray *transmission and* thickness of alveolar process J periodont Res 6 (1971), 152
— and LINDEN L Å Quantitative longitudinal study of enamel decalcification in vitro I A method for recording the course of demineralisation of ground enamel sections with an iodine 125 source Svensk tandläk -T 66 (1974) 69
HODGMAN D C Handbook of chemistry and physics Chemical Rubber Publishing CO Cleveland Ohio 1963
HOLLENDER L and LYSELL G Determination of *small* mass differences in roentgenography II Experimental investigations with a roentgen diffraction apparatus Acta radiol Ther Phys Biol 11 (1972) 145
JACOBSON B X ray spectrophotometry in vivo Amer J Roentgenol 41 (1964) 202
LYSELL G Determination of small mass differences in roentgenography III Experimental investigations with a dental roentgen apparatus Acta radiol Ther Phys Biol 12 (1973) 113
MENDENHALL W Introduction to linear models and the design and analysis of experiments Wadsworth Publ Co Belmont California 1968
OMNELL K Å Quantitative roentgenologic studies on changes in mineral content of bone in vivo Acta radiol (1957) Suppl No 148
SCHMELING P Measurement of bone mineral content using radiation sources An annotated bibliography AE 454 Studsvik 1972

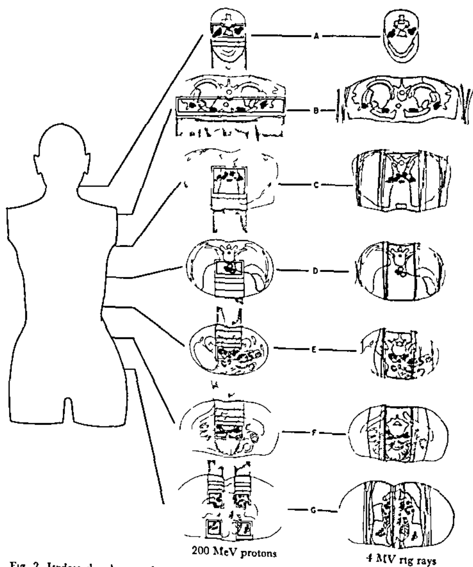


Fig 2 Isodose d

approximately locate the modified Bragg peak at depth t issue density This compensation is required in order to

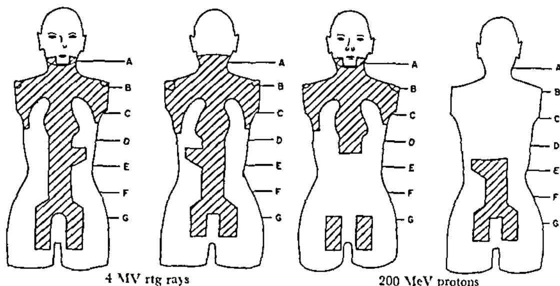


Fig 1 Projection on the of the parallel opposed mantle and inverted Y roentgen ray The posterior mantle and anterior inverted Y fields are not lth of the mantle field is about 11 cm in the neck 12 cm and 8 cm above and below the mediastinal region and along the inverted Y The limbs of the inverted Y and the axillary *fields are 4 cm wide The overall length was 71 cm* The thickness of the phantom varies from 15 cm in the neck to 22 cm in the pelvis

The isodose distribution for parallel opposed 4 MV roentgen-ray fields was determined using photographic methods

The isodose distribution for single fields using a 200 MeV proton beam altered to have a modified Bragg peak 4 to 8 cm deep was calculated using available physical data Corrections for variation in the range of protons caused by changes in tissue density of air-containing organs and bone were calculated and applied

Comparison of treatment plans

Isodose distribution The ability to confine the proton dose to a designated volume and to reduce the dose to normal tissues is evident in the isodose distribution displayed in Figs 2 to 4

The isodose distribution at 7 levels 5 cm apart for the parallel-opposed roentgen ray and single proton fields are illustrated in Fig 2 The 100 per cent isodose volume has been stippled

The proton and roentgen ray isodose distribution in the abdomen are compared in Fig 3 Full thickness of the abdomen irradiated with parallel opposed roentgen ray fields is contained within the 100 per cent isodose region However, with single proton fields only the designated node-containing volume lies within the

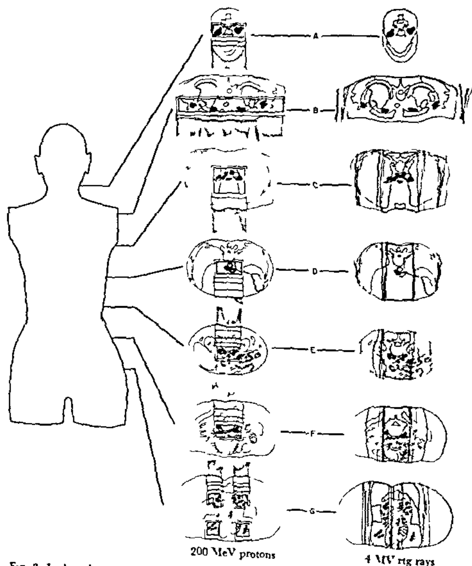


Fig. 2. [illegible] ... protons is limited ... 4 to 8 cm wide region representing the ... the cross-section indicate the use of a ... This compensation is required in order to

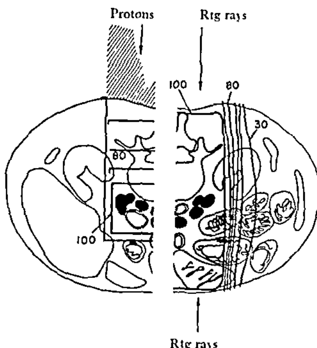


Fig 3 Isodose distribution for the 200 MeV proton and 4 MV roentgen ray fields side by side on these cross sections from the abdominal region A single proton field is used with a modified Bragg peak 4 cm wide to deliver the desired dose in the tumor containing region The overlying lumbar vertebrae receive a lower dose Intestinal volume beyond the Bragg peak is not irradiated For the roentgen irradiation plan in order to deliver the designated dose to the tumor containing region the tissues on either side receive the same dose This tissue sparing capacity of protons is well demonstrated in this type of presentation The hatched area designates the tissue density compensating bolus

100 per cent isodose region In the thorax the overlying mediastinum receives a decreasing dose, and the thoracic bone marrow is not irradiated In the abdomen the proton beam is incident on the back, and in this instance important volumes of bowel are not irradiated

The contrast in the dimensions of the area receiving 100 per cent of the dose between roentgen rays and protons is evident in Fig 4 The 100 per cent isodose region is diagrammatically displayed in the sagittal plane, note the large area of unirradiated tissue beyond the Bragg peak

Numerical index The normal tissue-sparing capacity of the proton dose distribution may be specified by determining the integral dose to normal tissues and by defining the volume of tissue not irradiated The integral dose to normal tissues is obtained by subtracting the tumor-containing volume integral dose from the total The tumor-containing volume integral dose is specified for roentgen rays as the dose to the volume contained within the proton 100 per cent isodose contour (Table 1)

A numerical index representing the degree of dose reduction may be obtained from the ratio of the roentgen ray and proton parameters (Table 2) The improved dose distribution available with proton irradiation permits a threefold reduction in the irradiated volume while achieving the desired dose in the tumor-containing volume For normal tissue this corresponds to an integral dose ratio protons/roentgen rays of 1/4 4 $\approx$ 0 23

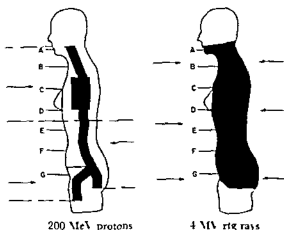


Fig 4 The extent of the 100% isodose volume across the phantom in the sagittal plane is represented diagrammatically for the 4 MV roentgen ray and 200 MeV protons This is actually a restatement of all the material presented in previous figures This region extends across the phantom when 4 MV roentgen rays are utilized and is represented by a central column for the 200 MeV protons The 100% isodose region is shown in the black The unirradiated areas are shown in white the areas receiving less than 100% are stippled The contrast existing between the irradiated volumes and integral dose between the protons and the roentgen rays is well represented in this presentation

Another suitable index showing the normal tissue sparing qualities of protons over roentgen rays is to specify the volume of tissue not irradiated by protons but which was irradiated with roentgen rays The unirradiated volume using the proton treatment plan is about one half of the roentgen ray treated volume As indicated above and displayed in Fig 4 this volume includes important volumes

Table I

Normal tissue parameters for total nodal irradiation

Radiation	Irradiated volume		Integral dose		
	Total (cm)	100% isodose region (cm³)	Total (g rad)	100% isodose region (g rad)	Normal tissue (g rad)
Rtg ray Parallel opposed mantle and inverted Y field	28 913	14 932	22 050	14 932	17 651
Proton Single mantle and inverted Y field	9 233	4 399	8 376	4 399	3 977

Table 2

Tissue sparing indices

Parameter	Ratio rtg ray/proton
Irradiated volume	3 1
100% isodose volume	3 4
Integral dose	2 6
Normal tissue dose	4 4

of bone marrow, bowel, skin and hair Such tissue sparing does have biologic *significance*

Discussion

It is recognized that this presentation deals with a hypothetical situation Practical considerations of tumor volume and beam localization must be perfected before proton therapy will become available

Tumor volume localization exists as a real problem, but it is not unique to proton therapy Existing methods utilizing surgical exploration, lymphangiography, radioactive imaging, ultrasound and diagnostic roentgen rays can be usefully exploited to define a tumor-containing volume with a reasonable margin of accuracy The degree of uncertainty in defining the tumor-containing volume can be compensated for by increasing the dimensions of the treatment field and *the width of the modified Bragg* peak

The single condition that must be accurately accounted for in each treament plan is the variation in tissue *densities Such variations affect* the range of protons in tissue and the location of the Bragg peak

The location of the proton beam may be established directly or indirectly Energetic particles generate radiation activity directly in cellular material through which they pass Oxygen-15 and carbon-11 are particularly suitable because of their relative abundance and short half lives These have been used with appropriate imaging technique to locate alpha particle beams in patients and in phantoms (MacCabe et *coll 1969*) *Calculations for 200 MeV protons indicate* a reasonable density of beam induced events from oxygen-15 production in tissue, with a total dose of about 20 rad (Bennett et coll) The location of a specified proton beam may be calculated if the tissue density distribution is known, this distribution may be determined from roentgenograms Some check on the calculations is effected by monitoring the dose with an ionization chamber

in the area of interest, and varying the energy of the particles These methods will allow definition of the beam with an error less than plus or minus one centimeter

Clinical perspective

Patients with Hodgkin's disease treated with 4 000 to 4 400 rad to the mantle and inverted Y experience considerable morbidity Frequently the course of therapy has to be interrupted between the mantle and inverted Y course of therapy in order to preserve tolerance Nausea, vomiting and lethargy are common complaints Skin pigmentation and hair loss occur. Peripheral blood parameters document a decrease in the bone marrow (KAPLAN 1972)

With proton irradiation there should be only a modest skin reaction of the incident field with an absorbed dose of 2 800 rad Epilation would not occur. Bone marrow reserve will be increased, since the thoracic vertebra will be spared Nausea and vomiting will be reduced because the major part of intestinal volume will not be irradiated The course of therapy can be reduced to one month since it is possible to irradiate the entire volume at one time

A real advantage to the patient derives from the bone marrow sparing If a recurrence of disease does occur following irradiation, the patient benefits because he has an increased bone marrow reserve Therefore, a full course of chemotherapy can be utilized if such therapy is indicated

Insufficient time has elapsed to determine what late changes total nodal irradiation will produce However, hepatic, cardiac, and pulmonary changes have been documented (KAPLAN 1972) In view of this a reduction in volume of normal tissue irradiated and a reduction in dose to these normal tissues that are irradiated would be advantageous

Total nodal irradiation or extended field irradiation is indicated for other malignant tumors (FLETCHER & RUTLEDGE 1972, VONGTAMA et coll 1974) However, such large field irradiation is not used for malignancies that require a therapeutic dose of about 6 000 *rad* because of normal tissue damage If the dose to normal tissues could be reduced the usefulness of total nodal or extended field irradiation could be determined for carcinoma of the bladder, testicle, cervix, *ovary and uterus with* para-aortic metastases Extended field irradiation of carcinoma of the bronchus, esophagus, pancreas and kidney should be *tried*

Conclusion The improved dose distribution obtained for 200 MeV protons over 4 MV roentgen rays for total nodal irradiation indicates that patients receiving this therapy would have less side *reactions and* morbidity

Table 2

Tissue sparing indices

Parameter	Ratio rtg ray/proton
Irradiated volume	3 1
100% isodose volume	3 4
Integral dose	2 6
Normal tissue dose	4 4

of bone marrow, bowel, skin and hair. Such tissue sparing does have biologic *significance*

Discussion

It is recognized that this presentation deals with a hypothetical situation Practical considerations of tumor volume and beam localization must be perfected before proton therapy will become available

Tumor volume localization exists as a real problem, but it is not unique to *proton therapy Existing methods utilizing surgical exploration,* lymphangiography, radioactive imaging, ultrasound and diagnostic roentgen rays can be usefully exploited to define a tumor-containing volume with a reasonable margin *of accuracy The degree of uncertainty in defining the tumor-containing volume* can be compensated for by increasing the dimensions of the treatment field and the width of the modified Bragg peak

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Conclusion The improved dose distribution obtained for 200 MeV protons over 4 MV roentgen rays for total nodal irradiation indicates that patients receiving this therapy would have less side reactions and morbidity

SUMMARY

The treatment plans and dose distribution for total nodal irradiation are derived and compared for 4 MV (Clinac) roentgen rays and a 200 MeV proton beam having a variable width modified Bragg peak The proton integral dose to normal tissues is 23 per cent of that received from roentgen rays, but the dose to the disease-containing volume is the same The proton dose can be delivered using single fields, whereas opposed fields are required for roentgen rays The sharp cut off of dose excludes important volumes of intestine and bone marrow from the irradiated volume which will be reflected in less patient morbidity

ZUSAMMENFASSUNG

Die Behandlungsplane und Dosisverteilung fur eine Totalbestrahlung der Knoten wird hergeleitet und fur 4 MV (Clinac) Rontgenstrahlen und 200 MeV Protonen Strahlen mit einem veranderlich weiten modifizierten Bragg Peak verglichen Die Protonen Integraldosis zum normalen Gewebe ist 23 Prozent der von Rontgenstrahlen erhaltenen Dosis, aber die Dosis des Volumens, das die Erkrankung enthalt, ist diegleiche Die Protonendosis kann unter Verwendung eines einzelnen Feldes verabfolgt werden, wahrend bei der Rontgenbestrahlung entgegengesetze Felder notwendig sind Die scharf abgeschnittenen Dosen schliessen bedeutungsvolle Volumina des Darmes und des Knochenmarks vom bestrahlten Volumen aus, was in einer geringeren Morbiditat der Patienten zum Ausdruck kommt

RÉSUMÉ

Les auteurs ont etabli et comparé les plans de traitement et les distributions de doses pour l'irradiation totale des ganglions pour des rayons de Roentgen de 4 MV (Clinac) et pour un faisceau de protons de 200 MeV ayant un pic de Bragg modifié a largeur variable La dose intégrale de protons aux tissus normaux est de 23 pour-cent de celle reçue par les irradiations aux rayons Roentgen, mais la dose au volume contenant le tissu malade est la même La dose de protons peut être délivrée en utilisant des champs uniques, alors que des champs opposes sont nécessaires pour les rayons Roentgen La delimitation nette de la dose permet d'exclure du volume irradié d'importants volumes d'intestin et de moelle osseuse, ce qui se reflete dans une moindre morbidité des malades

REFERENCES

ARC [illegible] of Proton radiation [illegible] lished February 1973

[illegible] n therapy Radiology 110 (1974), 445

BENNETT G W, GOLDBERG A. C, LEVINE G S, BALSAMO J C, GUTHY J H and ARCHAMBEAU J. O Beam localization via ^{15}O activation in proton radiation therapy To be published in Nucl Instr Meth

FLETCHER G H. and RUTLEDGE F N Extended field technique in the management of the cancers of the uterine cervix Amer J Roentgenol 114 (1972), 116

KAPLAN H S Hodgkin s disease Harvard Press Cambridge, Mass , 1972
KOEHLER A M and PRESTON W M Protons in radiation therapy Radiology 104 (1972), 191
MACCABEE H D, MADHVANATH U and RAJU M R Tissue activation studies with alpha particle beams Phys in Med Biol 14 (1969), 213
STENSON S Clinical experience with proton beams *In* Proc Symp Pion and Proton Radiotherapy, p 89 National Accelerator Laboratory, Batavia Ill 1971
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REFERENCES

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EXPERIMENTAL PARTICLE RADIATION THERAPY IN ANIMAL NEOPLASIA

II Alpha particles versus protons

S W LIPPINCOTT, J L MONTOUR and J D WILSON

The employment of monoenergetic beams of charged heavy particles for radiation therapy of certain human neoplasms has been tried in a limited number of institutions (FALKMER et coll 1962, TOBIAS et coll 1957) The theoretical advantages of particle radiation in comparison to standard forms of electro magnetic radiation have been thoroughly discussed in the literature (FALKMER et coll 1959, 1962, KOEHLER & PRESTON 1972, LIPPINCOTT et coll 1963, 1973 a, MOORE 1971, WITHERS 1973) Briefly, the physical factors associated with the desirable features are (1) control of depth of penetration of the beam, (2) use of a single Bragg peak to deliver an increased amount of energy to a given site (neoplasm), or use of successive multiple Bragg peaks to a given volume of target tissue with resultant essentially uniform ionization, (3) selection of particles with high LET (linear energy transfer) resulting in increased ioniza tion in the irradiated tissue in contrast to that of low LET gamma or roentgen irradiation, and (4) low OER (oxygen enhancement ratio) which means in a

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practical way that increasing degrees of hypoxia or even anoxia in the neoplasms do not decrease the efficiency of the particle radiation, as is generally considered to be the case for sparcely ionizing electromagnetic radiation

Preliminary investigations using particle radiation therapy have been carried out principally by the Uppsala and Berkeley groups (FALKMER et coll 1962, TOBIAS et coll 1957) This constitutes an outstanding achievement in coordinating the scientific talent of biomedical investigators with those of physicists and engineers The original difficulties in establishing such joint efforts were not from lack of rapport but because the devices for providing the particle beams were designed originally for experiments in physics. Adaptations and refinements in beam technology resulted in overcoming what were once considered limitations to such a type of endeavour Both protons and alpha particles have now been used and the forthcoming meson factory presumably will make available an even more desirable particle It is also quite possible that in the future sources of sufficiently energetic heavy ions will be available for therapy

In the radiation therapy of any neoplasm there are two well recognized problems, namely (1) the normal tissue tolerance to the quality of radiation to be used, since this limits the total dose that may be given and into what number of fractions the total dose should be administered over a prescribed period of time, and (2) employing a given type of radiation for the first time experimentally its RBE (relative biologic efficiency) has to be determined Usually, the latter is determined by comparing the tumoricidal effects of the quality of radiation under investigation with that for gamma or roentgen irradiation In regard to these two important factors, electrons, protons, deuterons and alpha particles have been used in our laboratories The normal tissue tolerance has been evaluated in short and long term follow up observations after radiation of the skin of mice, rabbits, monkeys and pigs (LIPPINCOTT et coll 1973 a) Using these same particles versus roentgen irradiation and electrons the RBE has been determined in a model system employing the C_3H/HeJ mammary neoplasm

The purpose of the present investigation was to compare the therapeutic efficiency in animal neoplasia of alpha particle versus proton radiation with the thought that such data will be of value to those who may plan to investigate the currently available as well as future sources of higher LET radiation Following alpha particle bombardment the density of ionization in tissue is four times that in tissue following proton radiation The model system used consisted of implanting a one mm cube of 100 per cent transplantable C_3H/HeJ mammary neoplasm into the thigh of the mouse at a selected depth and then determining with a wide range of single doses which form of radiation was most successful in completely destroying the neoplasms Probit analysis was used to



Fig. 1. Thirty days after irradiation with 65 MeV alpha particles (3 mm penetration). a) 3 000 rad b) 3 [illegible]00 rad c) 4 000 rad d) 4 [illegible]00 rad

fit a dose response curve. The χ^2 test indicated that each of the curves obtained adequately fitted the observed data. The ED$_{50}$ for 1 MeV electrons and 250 kV roentgen irradiation as well as the RBE for them has been previously reported (LIPPINCOTT et coll. 1973 b). The relative biologic efficiency for electrons compared to roentgen irradiation at the fifty per cent effect level (ED$_{50}$) is 1.73. The data being reported now are concerned with the RBE of alpha particles versus protons.

Beam calibration and dosimetry. The principles involved and the methodology for beam calibration and dosimetry have been described before (LIPPINCOTT et coll. 1973 a, b). For that reason only one example with the alpha particle will be cited herein. The alpha particle energy of the Oak Ridge Isochronous Cyclotron was chosen so that after the beam passed through the mylar vacuum window, the transmission ion chamber and the air path, the energy at the skin was sufficient to penetrate 3 mm of tissue. The rotating wheel with different foils was designed so that the dose depth curve was uniform. The transmission ion chamber was calibrated with the Faraday cup and the beam area determined by a lucite aperture. The uniformity over the area was measured by TLD chips of manganese activated calcium fluoride. The energy of the alpha particles was 73.5 MeV inside the cyclotron and 65 MeV at the skin surface. Thus the charge collected by the ion chamber gives the number of alpha particles per cm^2 and the average energy of the alpha particle gives the energy fluence. Since

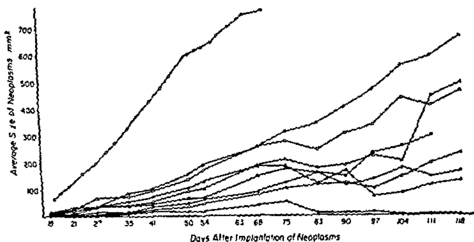


Fig 2 Growth curves of 1 mm³ tumor implants in non irradiated mice and in mice after 16 3 MeV proton irradiation ○ Control, ● 1 000, □ 1 500 ▲ 2 000, △ 2 500 ■ 3 000, ○ 3 500 ● 4 000 ◐ 4 500 rad

this fluence is stopped in a known depth with a uniform dose-depth, the energy deposited per gram is known and the current integrator can be set to stop the bombardment when the desired dose is achieved

Results

The tolerance of normal tissue to bombardment by alpha particles and by protons was determined by irradiating the thigh of the mouse, since in the therapeutic procedure the transplantable neoplasm was to be irradiated in that site In Fig 1 the skin of the thigh is shown thirty days after alpha bombardment with 3 000, 3 500, 4 000 and 4 500 rad respectively These doses are of interest because in a total of forty-seven mice receiving these doses only two animals had persisting tumor The effect then of radiation at these doses to the overlying normal tissue is of paramount importance The resultant scarring in the three lowest doses is rather minimal while for the highest dose it is quite prominent These animals were followed further clinically for another two months It was considered that the early lesions and the degree of fibrosis that followed were well within what could be considered as reasonable limits

In a previous publication (LIPPINCOTT et coll 1973 b), comparing the RBE of electrons versus roentgen irradiation, the rate of growth and time of death following implantation into the thigh of the mouse of a non irradiated 1 mm

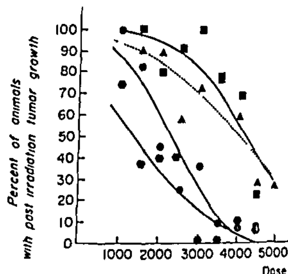


Fig 3 Dose (rad) response curves fitted by probit analysis ● alpha, ■ proton ● electron irradiation, ▲ roentgen irradiation

cube of tumor was established Several groups of animals receiving innoculations subcutaneously into the thigh had similar growth curves and were again similar The implantations by trochar were made so that the selected energies for both types of particles penetrated 3 mm and thus were capable of completely irradiating the 1 mm cube of tumor forty-eight hours after inoculation In Fig 2 a composite curve for growth of the non-irradiated tumor implants is shown

The data showing the results of proton irradiation on tumor growth are presented graphically at doses ranging from 1 000 to 4 500 rad in Fig 2 In animals whose neoplasms were not completely obliterated, proton irradiations delayed the appearance of the implanted tumors at all doses employed This delay was directly a function of radiation dose Of ninety-nine animals with implanted neoplasms irradiated with doses of protons ranging from 1 000 to 4 500 rad there was a failure of suppression of tumor growth in eighty However, Fig 2 does show that in spite of this very high percentage of failure to control tumor growth there was a distinct palliative effect as judged by post-irradiation time of survival With increasing dose when 4 500 rad was given ten out of thirteen neoplasms were destroyed

The result of irradiating one mm cubes of the mammary carcinoma transplanted into the dermis of the thigh of C_3H/HeJ mice is shown in Table 1 The objective was to determine the RBE of 65 MeV alpha particles compared to 16 3 MeV protons, both of which penetrated 3 mm The endpoint for this was the 50 per cent effect dose (ED_{50}) Dose response curves were fit to the data for each of these two types of particles by means of a probit analysis (Fig 3) Also

Table 1

Experimental particle radiation therapy in animal neoplasia 65 MeV alpha particles versus 16 3 MeV protons Transplanted C_3H/HeJ neoplasms in mice

Dose (rad)	65 MeV alpha particles			16 3 MeV protons		
	No irradiated	No with positive growth after irradiation	Per cent with positive growth after irradiation	No irradiated	No with positive growth after irradiation	Per cent with positive growth after irradiation
1 000	11	7	63	14	14	100
1 500	13	5	37	12	12	100
2 000	10	4	40	11	9	81
2 500	12	5	41	13	12	92
3 000	12	0	0	12	12	100
3 500	13	0	0	10	8	80
4 000	10	1	10	14	10	71
4 500	12	1	8	13	3	23

included are the dose response curves for low energy electrons and 250 kV roentgen rays previously reported (LIPPINCOTT et coll 1973 b) Chi square tests indicated no evidence of lack of fit for the probit fit curves ($p > 0\ 25$ for all except the proton data with p near 0 05) The estimated median effective doses (ED_{50}) and their standard errors are shown in Table 2 It is apparent that alpha particles are more effective than protons, roentgen rays or electrons The RBE for the alpha particle versus the proton is 3 16

Discussion

The reasons for anticipating that protons, alpha particles, negative pi mesons and heavy ions may offer advantages over conventional electromagnetic radiation for tumor therapy were cited earlier in this paper Since the earlier reports, several recent publications have offered further comments and data as to why these qualities of radiation should be fully explored therapeutically GRAFFMAN & JUNG (1970) state that the use of high energy protons in radiation therapy has been limited and hence the information is meager regarding their radiobiologic effects in therapy They further point out that from the experience in Uppsala that no clinical effects have been encountered which would indicate a marked dissimilarity in RBE between high energy protons and other low LET radiation It was also noted that superficial tissue seemed to tolerate well

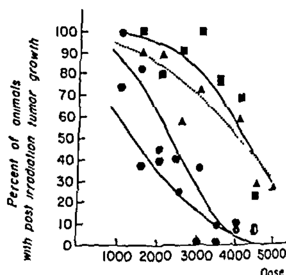


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tion but also distribution of atomic numbers, flux and energy of the various particles comprising the beam must be known They further state that heavy ion beams of wide energy spreed will allow irradiation of relatively large tumors with good distribution and high LET With accelerated heavy ions it should be possible to produce advantageous depth dose distributions similar to those obtained with pi mesons and with protons or alpha particles and much better than those obtained with gamma rays Concerning the important parameter of high LET, KAPLAN et coll (1973) have made the interesting comment that the RBE of high LET radiations may be expected to increase significantly when a given total dose is delivered in several temporally spaced fractions, due to the fact that there is little or no repair during the intervals between successive dose fractions of high LET radiation In contrast substantial repair occurs between successive fractionated exposures to low LET radiations

The need for further investigation of the efficiency of various types of heavy particles in radiation therapy seems well substantiated by the reports extant in the literature In successive stages towards the best plans for undertaking heavy particle radiation therapy for specific neoplasms in man it would appear that it is quite valuable to obtain prior normal tissue tolerance and RBE data for irradiated neoplasms in suitable animal species

SUMMARY

The median effective dose (ED_{50}) for alpha particle and proton radiation was determined in a model system in mice using a 100 per cent transplantable C_3H/HeJ mammary neoplasm Probit analysis was used to fit a dose response curve In both types of radiation the χ^2 test indicated that the curve obtained adequately fitted the observed data The estimated biologic efficiency (RBE) of alpha particle relative to proton radiation at the 50 per cent effect (ED_{50}) was 3 16

ZUSAMMENFASSUNG

Die mittlere effektive Dosis (ED_{50}) fur Alpha Partikel und Protonen Bestrahlung wurde an einem Modellsystem bei Mausen unter Verwendung eines 100 Prozent transplantablen C_3H/HeJ Mamma Neoplasmas bestimmt Es wurde eine Wahrscheinlichkeitsanalyse verwendet um eine Dosis-Respons Kurve darzustellen. Bei beiden Strahlentypen zeigte der χ^2 Test dass die erhaltenen Kurven die beobachteten Daten gut wiedergaben. Die berechnete relative biologische Effektivitat (RBE) der Alpha Partikel gegenuber der Protonen Bestrahlung bei einem 50 Prozent Effekt (ED_{50}) betrug 3,16

RÉSUMÉ

La dose effective mediane (ED_{50}) pour les particules alpha et le rayonnement protonique a été determinee sur un système de modèle sur des souris au moyen d un neoplasme mam maire transplantable à 100 pour cent C_3H/HeJ L analyse des probits a été utilisee pour

Table 2

ED_{50} and RBE values for each type of irradiation

Median effective dose (ED_{50})		
Particle	ED_{50}	SE
16.3 MeV proton	4.277	564
250 kV roentgen irradiation	4.014	555
1 MeV electron	2.326	203
65 MeV alpha particle	1.352	522
Relative biologic efficiency (RBE) at ED_{50}		
		RBE
Proton relative to roentgen irradiation		0.94
Electron relative to roentgen irradiation		1.73
Alpha particle relative to roentgen irradiation		2.97
Electron relative to proton		1.88
Alpha particle relative to proton		3.16
Alpha particle relative to electron		1.67

a constant-dose proton field. They concluded that high energy protons offer an outstanding flexibility in the shaping of the dose distribution. The beam cross section as well as the surface of maximum penetration of the field can be formed easily to an intricate shape by appropriate diaphragms and range determining moulds. The depth-dose distribution can be varied continuously from one of the Bragg type to one of constant dose.

ARCHAMBEAU et coll. (1974) also state that no radiobiologic advantage can be claimed for protons per se. Yet, according to them, the ability to confine the proton dose to a designated volume provides greater flexibility and resourcefulness for proton radiation therapy than does roentgen ray therapy for dose delivery to the neoplasm. The ability to confine the major fraction of proton absorbed dose to a designated volume allows the decrease of dose to normal tissue or the increase of dose to the neoplasm. Localization of the proton beam can be achieved by calculation and by appropriate tissue absorption techniques in which the proton beam is ranged through the tissue. In addition, tissue activation can be employed with appropriate detecting devices and computer analysis of the data to define the position of the Bragg peak. To support these statements, these authors have carefully reviewed the physical and radiologic status of proton therapy in the literature to date to which they have added an interesting comparative analysis for suggested proton and roentgen irradiation plans.

In pretherapeutic investigations with accelerated heavy ions TOBIAS (1973) has reported that to predict the RBE of heavy ions that not only dose distribu-

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FSTIMATION OF ABSORBED DOSE IN THYROIDS AND GONADS OF SURVIVORS IN HIROSHIMA AND NAGASAKI

T HASHIZUME, T MARUYAMA, K NISHIZAWA and A NISHIMURA

Analyzing the late effects of radiation from the atomic bombs in Hiroshima and Nagasaki is important for obtaining information on the possible human radiation injury after short term exposure The somatic effects within the life-time of the survivors such as inductions of cataract, leukemia and thyroid carcinoma have been analyzed (COGAN et coll 1949, AMAMOTO et coll 1960, HOLLINGSWORTH et coll 1963) The survivors and their posterity worry about the possibility of undesirable genetic effects Unfortunately, it is impossible to detect quantitatively the genetic injury for the relatively short time since exposure However, the extensive genetic investigations on the mouse (RUSSELL 1965 a, 1965 b), seem to provide relevant information that enables an estimate of *possible genetic mutations in man In addition*, physical calculations have provided a knowledge of in air tissue absorbed dose from the atomic bombs in Hiroshima and Nagasaki (AUXIER et coll 1966, HASHIZUME et coll 1967) For the purpose of assessing the dose response, the biologically significant dose is not

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ajuster une courbe de réponse de dose Pour ces deux types de radiation le test χ^2 a montre que la courbe obtenue s ajuste convenablement au résultat observé L efficacite biologique relative estimée (RBE) des particules alpha par rapport au rayonnement protonique pour l'effet 50 pour cent (ED_{50}) a été de 3,16

REFERENCES

ARCHAMBEAU J O, BENNETT G W, LEVINE G S, COWEN R and AKANUMA A Proton radiation therapy Radiology 110 (1974), 445

BARENDSEN G W, KOOT C J, VAN KERSEN B D, FIELD S B and PARNELL C J The effect of oxygen on impairment of the proliferative capacity of human cells in culture by ionizing radiation of different LET Int J Radiat Biol 10 (1966), 317

BENDER R S and DARDEN E B Jr A broad beam biology facility for proton irradiation at the Oak Ridge Isochronous Cyclotron Radiat Res 41 (1970), 247

FALKMER S, LARSSON B and STENSON S Effects of single dose proton irradiation of normal skin and Vx2 carcinoma in rabbit ears Acta radiol 52 (1959), 217

— FORS B, LARSSON B, LINDELL A, NÄSLUND J and STENSON S Pilot study on proton irradiation of human carcinoma Acta radiol 58 (1962), 33

GRAFFMAN S and JUNG B Clinical trials in radiotherapy and the merits of high energy protons *Acta radiol Ther Phys Biol 9 (1970), 1*

JANSEN C R, BAKER C P, CALVO W, RAI K R and LIPPINCOTT S W A method of effectively delivering multiple Bragg peaks in depth in the path of charged heavy particles in tissue, USAEC *report BNL-5538, Upton, NY*, Brookhaven National Laboratory, Atomic Energy Commission, 1960

KAPLAN H S, SCHWETTMAN H A, FAIRBANK W M, BOYD D, BAGSHAW M A *A hospital-based* superconducting accelerator facility for negative Pi-Meson beam radiotherapy Radiology 108 (1973), 159

KOEHLER A M and PRESTON W M Protons in radiation therapy Comparative dose distribution for protons, photons and electrons Radiology 104 (1972), 191

LIPPINCOTT S W, MOORE W H JESSEPH J E CALVO W and FARR L E Effects of heavy high energy charged particles I Literature review of effects of protons, deuterons and alpha particles in mammals with a theroretical consideration of the negative Pi Meson Arch Path 76 (1963), 497

— MONTOUR J L, WILSON J D, BENDER R and BILEK F (a) Normal tissue tolerance to particle and heavy ion radiation Arch Path 96 (1973), 27

— — — COHN S and FLORA R E (b) *Experimental particle radiation therapy* in animal neoplasia I Electron versus roentgen irradiation Acta radiol Ther Phys Biol 12 (1973), 541.

MOORE W H The application of 200 MeV protons to biomedical research Brookhaven National Laboratory, Informal Report BNL 16185, Aug 1971

TOBIAS C A Pretherapeutic investigations with accelerated heavy ions Radiology 108 (1973), 145

— ANGER H O and LAWRENCE J H Radiological use of high energy deuterons and alpha particles Amer J Roentgenol 67 (1957), 1

WITHERS H R Biological basis for high-LET radiotherapy Radiology 108 (1973), 131

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ESTIMATION OF ABSORBED DOSE IN THYROIDS AND GONADS OF SURVIVORS IN HIROSHIMA AND NAGASAKI

T HASHIZUME, T MARUYAMA, K NISHIZAWA and A NISHIMURA

Analyzing the late effects of radiation from the atomic bombs in Hiroshima and Nagasaki is important for obtaining information on the possible human radiation injury after short term exposure The somatic effects within the lifetime of the survivors such as inductions of cataract, leukemia and thyroid carcinoma have been analyzed (COGAN et coll 1949, AMAMOTO et coll 1960, HOLLINGSWORTH et coll 1963) The survivors and their posterity worry about the possibility of undesirable genetic effects Unfortunately, it is impossible to detect quantitatively the genetic injury for the relatively short time since exposure However, the extensive genetic investigations on the mouse (RUSSELL 1965 a, 1965 b), seem to provide relevant information that enables an estimate of possible genetic mutations in man In addition, physical calculations have provided a knowledge of in air tissue absorbed dose from the atomic bombs in Hiroshima and Nagasaki (AUXIER et coll 1966, HASHIZUME et coll 1967) For the purpose of assessing the dose response, the biologically significant dose is not

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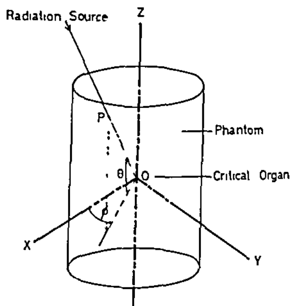


Fig 1 Arrangement for phantom irradiation X—Y plane = horizontal plane of phantom, X—Z plane = sagittal plane of phantom, O = critical organ, P = incident point of radiation on phantom Θ = latitudinal angle, Φ = longitudinal angle

the in-air tissue absorbed dose but the absorbed dose in critical organs with respect to carcinomas and genetic effects An estimation of organ absorbed dose from the in-air tissue absorbed dose, necessitates a determination of the relationship between these two types of dose This relationship can be replaced with the ratio of the absorbed dose in critical organs to in-air tissue absorbed dose (this ratio is termed hereafter as RATA)

The present report presents an estimation of RATA in laboratory measurements and of the absorbed dose in the thyroids and gonads (ovaries and testes) as a function of distance from the hypocenter in Hiroshima and Nagasaki, using previous data on in-air tissue absorbed dose (HASHIZUME et coll 1967) Since some scattering of radiations from the atomic bombs occurs in air from the point of detonation it is considerably complicated (RITCHIE & HURST 1959) The angular distribution was taken into account for the dose estimation of critical organs Tissue-equivalent phantoms instrumented for gamma-ray and neutron dose measurements were exposed to simulated radiation sources for the initial gamma-rays and neutrons from the atomic bombs, so that the RATA at the thyroids and gonads were determined as a function of incident angles of monodirectional radiation beams to the survivors The correction of angular distributions for the RATA was carried out by multiplying the RATA experimentally determined for an incident angle by the fraction of radiations entering the survivor from the atomic bombs at the corresponding angle, and by summing up the products over all angles The absorbed dose in the critical organs due to

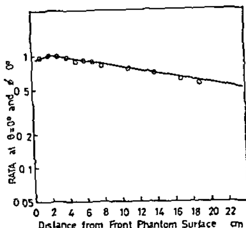


Fig 2 Ratio of absorbed dose in tissue equivalent phantom to in air tissue absorbed dose (gamma rays) Open circles represent measured values with a thermoluminescent dosimeter This curve was determined as a function of depth in the phantom for incident radiation beams at $\Theta = 0°$ and $\Phi = 0°$

neutrons was separated into three parts, namely recoil proton dose from elastic collision with hydrogen nuclei, gamma ray dose from $^{1}H(n,\gamma)^{2}D$ reaction and proton dose from $^{14}N(n\ p)^{14}C$ reaction

Materials and Methods

The simulated gamma ray and neutron sources for the atomic bombs were 12 MV roentgen rays produced by a medical betatron and neutrons from the $^{9}Be(d\ n)^{10}B$ reaction by bombarding a thick beryllium target with 2 5 MeV deuterons accelerated by a Van de Graaff accelerator The feasibility of using these sources has been investigated by comparisons between the energy spectra and characteristics of the depth dose curves for these simulated sources and those for the atomic bombs (HASHIZUME *et coll* 1973)

The phantom used for roentgen ray dose measurements was an average woman Rando phantom which corresponded to a complete body 163 cm tall by adding phantoms of extremities made of M 3 materials (composition in wt % paraffin 76 5, MgO 22 4 and $CaCO_3$ 0 7, MARKUS 1956) This phantom, although approximately tissue equivalent for gamma rays, does not have the appropriate atomic composition to allow tissue equivalence for neutrons For this reason, the phantom used for neutron dose measurements was a Remab phantom (Rem 120 manufactured by Alderson Co, 165 cm in height) filled with the tissue equivalent liquid (composition in wt % water 56 9, glycerol 28 4, urea 7 6 and sucrose 7 1, ROSSI 1956) The lungs of the phantom were filled with

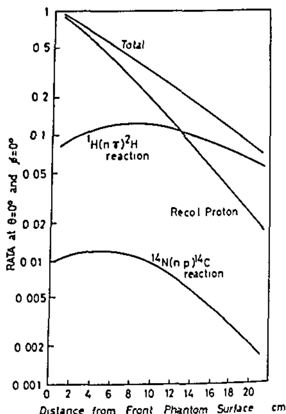


Fig 3 Ratio of absorbed dose in tissue-equivalent phantom to in air tissue absorbed dose (neutrons) The curve for recoil protons was measured with paired chambers The curves for $^1H(n\gamma)$-D reaction and for $^{14}N(n p)^{14}C$ reaction were calculated from thermal neutron distributions determined with a BF_3 proportional counter The curve for total gives the sum of ratios for recoil protons gamma rays from the $^1H(n\gamma)$-D reaction and protons from the $^{14}N(n,p)^{14}C$ reaction These curves were determined as a function of depth in the phantom for incident radiation beams at $\Theta = 0°$ and $\phi = 0°$

powder of tissue-equivalent plastics (Hiraoka et coll 1973) so that density of the lung was 0 3 g/cm³ These phantoms can be used for the standard Japanese body (163 cm in height and 55 kg in weight)

The 12 MV roentgen ray dose was measured with a thermoluminescent dosimeter (manufactured by Dai Nippon Toryo Co) made of thin glass tubing, formed into a right cylinder 1 mm in diameter and 6 mm long, filled with powder of Mg_2SiO_4(Tb) For neutron dose measurements, the paired chambers, which consisted of polyethylene-ethylene and carbon-CO_2 chambers, were used to measure the absorbed dose due to recoil protons from fast neutron elastic collisions Each chamber had an external diameter of 1 cm and a length of 3 cm This paired chamber technique has been reported by Matsuzawa et coll (1969) and Hiraoka et coll (1971) The BF_3-proportional counter which has an external diameter of 0 8 cm and a length of 6 cm was used to determine thermal neutron distributions in a phantom in order to estimate the absorbed dose due to protons and gamma-rays from the $^{14}N(n,p)^{14}C$ and $^1H(n,\gamma)$-D reaction by thermalization of neutrons within the phantom For the measurements of thermal neutrons, the phantom was an elliptically cyrindrical polyethylene

Table 1

The RATA, R (Θ, Φ), for ovaries Θ and Φ represent the latitudinal and longitudinal angles, respectively, as illustrated in Fig 1

Φ/Θ	−90°	−60°	−45°	−30°	0°	30°	45°	60°	90°
Gamma rays									
0°	0 15	0 62	0 72	0 76	0 76	0 74	0 64	0 51	0 23
30°	0 15	0 71	0 76	0 74	0 71	0 64	0 63	0 60	0 23
45°	0 15	0 68	0 69	0 75	0 67	0 63	0 60	0 54	0 23
60°	0 15	0 64	0 61	0 71	0 63	0 61	0 60	0 57	0 23
90	0 15	0 40	0 48	0 52	0 60	0 61	0 58	0 50	0 23
120	0 15	0 41	0 51	0 53	0 64	0 64	0 62	0 62	0 23
135°	0 15	0 47	0 59	0 56	0 68	0 72	0 60	0 64	0 23
150	0 15	0 63	0 63	0 64	0 72	0 72	0 71	0 71	0 23
180	0 15	0 64	0 67	0 69	0 73	0 72	0 70	0 62	0 23
Neutrons (recoil protons)									
0°	0 070	0 11	0 14	0 20	0 19	0 14	0 13	0 041	0 040
30	0 070	0 050	0 086	0 14	0 13	0 15	0 13	0 11	0 040
45°	0 070	0 025	0 080	0 096	0 092	0 10	0 077	0 074	0 040
60	0 070	0 019	0 026	0 051	0 053	0 054	0 031	0 028	0 040
90°	0 070	0 025	0 037	0 068	0 035	0 022	0 041	0 034	0 040
120°	0 070	0 012	0 029	0 063	0 066	0 067	0 043	0 040	0 040
135	0 070	0 034	0 059	0 11	0 10	0 11	0 069	0 13	0 040
150	0 070	0 090	0 12	0 18	0 18	0 19	0 18	0 20	0 040
180	0 070	0 18	0 10	0 10	0 19	0 17	0 20	0 11	0 040

container of 32 5 cm × 20 cm cross section and 60 cm high filled with the tissue-equivalent liquid

The positions of the critical organs were assumed to be as follows (a) the thyroids are 0 9 cm below the anterior surface of the neck phantom, (b) the more common locations of the ovaries are 5 cm part laterally from the minor axis of the torso phantom, and 10 cm from the front of the phantom and 10 cm from the back, although the positions may vary among individuals, (c) the testes are 0 7 cm deep on the minor axis of the torso phantom For the Rando phantom, a lucite cylinder, 1 5 cm in diameter and 2 cm high, was used to simulate the testes Lungs, ovaries, testes and the thyroids were provided as part of the Remab phantom The paired chambers and thermoluminescent dosimeters were placed at these positions in the phantoms and were exposed to the radiation sources In order to simplify correction of the RATA for the angular distributions, these ratios were determined as a function of the incident angles The

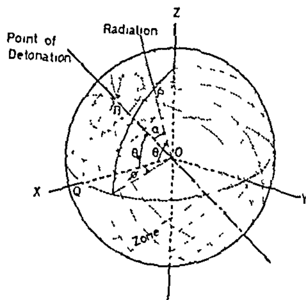


Fig 4 Schematic representation of globe system and laboratory system

phantoms were irradiated with various angles of incidence in terms of a latitudinal angle Θ and longitudinal angle Φ, which were taken to be $\Theta = 0°$ and $\Phi = 0°$ in a direction toward the normal line to the front surface of the phantoms (Fig 1) Each critical organ was placed on the central axis of the beam and was exposed to the 0° direction beams at a distance of 150 cm from the targets to the surface of the phantoms with the field size of about 50 cm × 50 cm

The absorbed doses from the $^{1}H(n,\gamma)^{2}D$ and $^{14}N(n,p)^{14}C$ reaction at the positions of the critical organs were calculated from the thermal neutron distributions measured with the BF_3-proportional counter, using a method proposed by Smith & Boot (1961) Similarly, central depth-dose curves for gamma-rays and protons from these reactions were determined

Results

Determinations of RATA Since all measurements were carried out for point sources, the absorbed doses measured were corrected for the inverse-square effect of distance between the target and the center of the detector As illustrated in Fig 2, an initial freehand smooth curve, based on measuring with the Farmer sub standard dosimeter, was fitted to the points plotted in terms of the values measured by the thermoluminescent dosimeter Fig 3 gives the RATA at $\Theta = 0°$ and $\Phi = 0°$ as a function of depths in the elliptically cylindrical phantom The RATA for neutrons was given for dose components from recoil protons, gamma-rays and protons The fraction of dose from the $^{1}H(n,\gamma)$ D reactions was

Table 2

The RATA for critical organs, $R(\Theta_0, \Phi_0)$, at $\Phi_0 = 0°$ The survivors are supposed to be exposed to radiation, facing the point of detonation These ratios were corrected for the angular distributions of radiation from the atomic bomb Distances from the hypocenter Hiroshima 590 m at 45°, 1 022 m at 30° and 2 201 m at 15°, Nagasaki 500 m at 45°, 866 m at 30° and 1 887 m at 15°

Organ	Θ_0	Gamma rays		Neutrons		
		Hiroshima	Nagasaki	Recoil	$^1H(n,\gamma)^2D$	$^{14}N(n,p)^{14}C$
Ovary	45°	0 62	0 62	0 097	0 095	0 0061
	30°	0 64	0 64	0 10	0 10	0 0062
	15°	0 65	0 66	0 11	0 10	0 0062
Testis	45°	0 86	0 87	0 59	0 095	0 013
	30°	0 87	0 88	0 60	0 095	0 013
	15°	0 87	0 88	0 62	0 095	0 013
Thyroid	45°	0 80	0 81	0 41	0 10	0 011
	30°	0 81	0 82	0 43	0 10	0 011
	15°	0 82	0 83	0 46	0 10	0 011

found to be predominant at the depth of 12 cm or more Proton dose from the $^{14}N(n,p)^{14}C$ reaction would also be important for estimations of the relative biologic effectiveness, although the fraction of proton dose was small compared with the gamma ray dose from the $^1H(n,\gamma)^2D$ reaction

As an example of the experimental results, the RATA of an ovary for the 12 MV roentgen rays and recoil protons is given in Table 1 for various latitudinal angles Θ and various longitudinal angles Φ For the RATA of gamma rays from the $^1H(n,\gamma)$ D [illegible] lculated $\Theta = 0°$ and Φ

Correction o [illegible] axis in the direction o [illegible] The X—Y plane represents the horizontal plane on the laboratory system on which the experiments were carried out X axis corresponds to the horizontal line on the anterior abdomen of the survivors, when they were exposed to radiation facing to the point of the detonation A point P represented by an angular *co-ordinate* (α, β) on the globe system is given by a co-ordinate (Θ, Φ) on the laboratory system The angle Θ_0 represents the angle between the horizontal line on the anterior abdomen of the survivors and the direction toward the detonation point and Φ_0 is the angle between the horizontal line on the anterior abdomen of the survivors and the direction toward the hypocenter

Table 3

The RATA for critical organs, $R(\Theta_0, \Phi_0)$, at the incident angle of 30° (distance from the hypocenter, Hiroshima 1 022 m, Nagasaki 866 m). Φ_0 represents the angle between the normal line to the front surface of the survivors and the direction toward the hypocenter

Organ	Φ_0	Gamma rays		Neutrons		
		Hiroshima	Nagasaki	Recoil protons	$^1H(n,\gamma)^2D$	$^{14}N(n,p)^{14}C$
Ovary	0°	0.64	0.64	0.10	0.10	0.0062
	45°	0.62	0.63	0.092	0.095	0.0054
	90°	0.62	0.62	0.081	0.090	0.0050
	135°	0.64	0.65	0.10	0.095	0.0056
	180°	0.65	0.66	0.11	0.10	0.0061
Testis	0°	0.87	0.88	0.60	0.095	0.013
	45°	0.84	0.85	0.55	0.095	0.013
	90°	0.71	0.70	0.40	0.095	0.013
	135°	0.68	0.67	0.30	0.095	0.019
	180°	0.72	0.72	0.30	0.095	0.025
Thyroid	0°	0.81	0.82	0.43	0.10	0.011
	45°	0.81	0.81	0.39	0.10	0.011
	90°	0.80	0.80	0.32	0.11	0.011
	135°	0.76	0.76	0.24	0.11	0.010
	180°	0.75	0.75	0.22	0.11	0.0099

The surface of the globe having an axis directed to the point of detonation was divided into 648 sections by drawing the latitudinal lines at intervals of 10° and the longitudinal lines at each 10°. The fraction of radiation entering the zone surrounded by two adjoining latitudinal lines, $f(\Theta_0,\alpha,\beta)$, for a given incident angle has been determined using the angular distribution of radiation from the atomic bomb (CHEKA 1966).

The RATA, $R(\Theta,\Phi)$, which was determined experimentally as a function of the angle Θ and Φ were converted into the angular co-ordinate on the globe system, $(R(\alpha,\beta)$ (HASHIZUME et coll. 1973). For a given angle Φ_0, the RATA (Θ_0,Φ_0) was obtained from the following equation

$$R(\Theta_0, \Phi_0) = \sum_{\alpha} \sum_{\beta} f(\Theta_0, \beta_i) \; R(\alpha,\beta) \qquad (1)$$

Thus, the RATA $R(\Theta,\Phi)$ was corrected for the angular distribution of radiations from the atomic bombs.

Table 4

The absorbed dose in critical organs for the initial gamma rays and neutrons in Hiroshima and Nagasaki

Organ	Distance (m)	Gamma rays	Neutrons		
			Recoil protons	$^{1}H(n,\gamma)^{2}D$	$^{14}N(n,p)^{14}C$
Hiroshima					
Ovary	500	1 720 rad	250 rad	247 rad	15 9 rad
	1 000	182	15 5	15 0	0 930
	1 500	18 7	0 954	0 900	0 056
	2 000	1 81	0 059	0 055	0 003
Testis	500	2 400	1 520	247	33 8
	1 000	247	89 3	14 3	1 95
	1 500	25 2	5 49	0 855	0 117
	2 000	2 44	0 338	0 053	0 007
Thyroid	500	2 230	1 050	260	28 6
	1 000	231	64 5	15 0	1 65
	1 500	23 6	3 97	0 900	0 099
	2 000	0 560	0 250	0 055	0 006
Nagasaki					
Ovary	500	4 480	84 3	81 7	5 25
	1 000	539	6 51	6 40	0 384
	1 500	78 0	0 482	0 450	0 028
	2 000	9 90	0 040	0 037	0 002
Testis	500	6 270	507	81 7	11 2
	1 000	808	37 4	5 89	0 806
	1 500	106	2 75	0 428	0 059
	2 000	13 2	0 230	0 035	0 002
Thyroid	500	5 730	353	86 0	9 46
	1 000	754	27 0	6 20	0 682
	1 500	98 8	2 03	0 450	0 050
	2 000	12 4	0 172	0 037	0 004

The RATA $R(\Theta_0, \Phi_0)$ for ovary, testis and the thyroid are given in Tables 2 and 3 Values of Θ_0 for corresponding distances were calculated, assuming that the height of the detonation point in Hiroshima and Nagasaki was 590 and 500 m respectively The slight differences in the RATA $R(\Theta_0 \ \Phi_0)$ in Hiroshima and Nagasaki are due to the differences of the height between both cities

Dose estimations In order to simplify the dose estimations it was assumed that the survivors were in a standing posture during the exposure The absorbed

Table 5

In-air tissue absorbed dose (HASHIZUME *1967*)

Distance (m)	Hiroshima		Nagasaki	
	Gamma-rays	Neutrons	Gamma rays	Neutrons
500	2 800 rad	2 600 rad	7 200 rad	860 rad
1 000	285	150	920	62
1 500	29 0	9 00	120	4 50
2 000	2 80	0 550	15 0	0 370

dose in the critical organs at the angles of Θ_0 and Φ_0, $D(\Theta_0, \Phi_0)$, was *given by* the following equation

$$D(O_0, \Phi_0) = D_{air}(O_0)\ R(\Theta_0, \Phi_0) \quad (2)$$

where $D_{air}(\Theta_0)$ is the in-air tissue absorbed dose at the point of the angle Θ_0

In the previous report (HASHIZUME et coll 1967), the in-air tissue absorbed dose *of the initial gamma-rays and neutrons have been estimated* as a function of distance form the hypocenter (Table 5) The absorbed dose in ovary, testes and the thyroid (using the in-air tissue absorbed dose data) for the initial *gamma-rays and neutrons obtained from equation (2) is given in* Table 4 for various distances from the hypocenter

Discussion

The RATA, namely the ratio of the absorbed doses in the critical organs to the in-air tissue absorbed doses, are the most fundamental quantities for the dose estimation in the critical organs The ratios varied irregularly with changes *of the angles Θ and Φ The RATA of an ovary for recoil protons ranged* from a minimum value of 0 012 *to a maximum* value of 0 20 The variations of the RATA with the angles of Θ and Φ may be due to the attenuation characteristics of neutrons in the irregularly shaped body

For recoil protons, $R(\Theta_0, \Phi_0)$ *for testis and the thyroid* were about five times as large as those for ovary, but for the gamma-rays from the $^1H(n,\gamma)$ D reaction they were only slightly different from organ to organ (Tables 2, 3) For both *the initial gamma-rays and neutrons, these values varied according* to the angle between the normal line to the front surface of the survivors and the direction toward the hypocenter, and increased with increasing the distance from the hypocenter *For an ovary,* the RATA *of recoil protons* was comparable with that of the gamma-rays from the $^1H(n,\gamma)$-D reaction

For an ovary, 49 per cent of the contribution to neutron dose comes from recoil protons with 48 per cent from the $^{1}H(n,\gamma)^{2}D$ reaction and with 3 per cent from the $^{14}N(n,p)^{14}C$ reaction In contrast to this, for the thyroid and testis, 78 to 84 per cent of the contribution to neutron dose comes from recoil protons and the $^{14}N(n p)^{14}C$ reaction with 14 to 19 per cent from the $^{1}H(n,\gamma)^{2}D$ reaction These results are of importance for the analysis of biologic effects induced by neutrons from the atomic bombs in Hiroshima and Nagasaki

When the exposed survivors were facing the point of detonation, the RATA, $R(\Theta_0, \Phi_0)$ for testis and the thyroid (Table 3) may have yielded an overestimation for other orientation of the survivors In both cities, the ratios of the total neutron dose to the total initial gamma ray dose for testis and the thyroid were about twice as large as the ratios for an ovary In Hiroshima, the ratio for each organ was about ten times as large as the corresponding ratio in Nagasaki These differences can be due to characteristics of the atomic bombs in Hiroshima and Nagasaki

The absorbed dose in an ovary was slightly less than the dose in the fetal head at the 4th month of gestation (HASHIZUME et coll 1973) This may be due to the fact that an ovary lies in deeper region than the fetal head the female body

In determining the RATA $R(\Theta, \Phi)$, the accuracy for measurements of absorbed dose by the thermoluminescent dosimeters and the paired chambers was found to be 3 per cent Variations in determinations of the critical organ positions were estimated to be 3 per cent Since the precise neutron energy in the phantom was unknown the choice of correction factor for neutron sensitivity of the carbon CO_2 chamber may introduce the error in the assessment of neutron dose of approximately 10 per cent at the maximum (NBS Handbook 1961) Furthermore, when the gamma ray dose in the phantom is high, additional errors of approximately 10 per cent may be introduced to the assessment of neutron dose, because in this case the neutron dose was evaluated as a result of subtraction of two nearly equal values Thus total errors in the determinations of the RATA for the initial gamma rays and recoil protons are estimated to be 6 and 26 per cent, respectively Errors in the estimation of the *in air* tissue absorbed dose in Hiroshima and Nagasaki have been less than 11 per cent for the initial gamma rays and less than 15 per cent for neutrons (HASHIZUME et coll 1967) The total errors in the estimation of absorbed dose in the critical organs by the present method have been about 13 per cent for the initial gamma rays and 30 per cent for neutrons, although uncertainties concerning energy and angular distributions of radiations released from the atomic bombs still prevail

The accuracy of the BF_3-proportional counter for measurements of thermal neutron fluence in a standard pile was reported to be 3 per cent by the calibration of Japan Electrotechnical Laboratory (TERANISHI 1966) The accuracy

of dose calculations for gamma-rays and protons from the $^{1}H(n,\gamma)D$ and $^{14}N(n,p)^{14}C$ reaction depends on systematic as well as on the statistical errors, namely the errors in the nuclear data used, estimated to be less than 6 per cent, the variations in the distance from the target to the counter, presumably less than 2 per cent, and the statistical error of 2 per cent in counting Thus the maximum error in the determinations of RATA for protons and gamma-rays from neutron reactions may be calculated to be 13 per cent, including errors of determinations of the in-air tissue absorbed dose with the paired chambers The total errors in the dose estimations for protons and gamma-rays were estimated to be about 20 per cent

Conclusion

The RATA $R(\Theta_0, \Phi_0)$ of an ovary ranged from a minimum value of 0 62 to a maximum value of 0 66 for the initial gamma-rays, 0 081 to 0 11 for recoil protons, 0 090 to 0 10 for gamma-rays from $^{1}H(n,\gamma)^{2}D$ reactions and 0 0050 to 0 0062 for protons from $^{14}N(n,p)^{14}C$ reaction, depending on a distance from the hypocenter and the posture of the survivors at the explosion time For testis, the RATA ranged from 0 67 to 0 88 for the initial gamma-rays, 0 30 to 0 60 for recoil protons, 0 013 to 0 025 for protons from the $^{14}N(n,p)^{14}C$ reaction and was 0 095 for gamma-rays from the $^{1}H(n,\gamma)^{2}D$ reaction

The absorbed dose in an ovary at 1 000 m from the hypocenter in Hiroshima was estimated at 182 rad for the initial gamma-rays, 15 5 rad for recoil protons, 15 rad for gamma-rays from the $^{1}H(n,\gamma)D$ reaction and 0 93 rad for protons from the $^{14}N(n,p)^{14}C$ reaction Corresponding absorbed dose in Nagasaki was 539 rad for the initial gamma-rays, 6 5 rad for recoil protons, 6 4 rad for gamma-rays and 0 38 rad for protons

Acknowledgement

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SUMMARY

Ratios of the absorbed dose in the thyroid and gonads to in air tissue absorbed dose were determined using simulated radiation sources for the initial gamma rays and neutrons from the atomic bombs The absorbed dose in these critical organs of the survivors exposed to gamma rays and neutrons from the 1945 atomic bombs in Hiroshima and Nagasaki was estimated as a function of distance from the hypocenter

ZUSAMMENFASSUNG

Verhaltniswerte der absorbierten Dosis in der Thyreoidea und den Gonaden zur in Luft Gewebe absorbierten Dosis wurden unter Verwendung simulierter Strahlenquellen fur die ursprunglichen Gamma Strahlen und Neutronen von den Atombomben bestimmt Die absorbierte Dosis in diesen kritischen Organen der Überlebenden, die den Gamma Strahlen und Neutronen der Atombomben von Hiroshima und Nagasaki ausgesetzt worden waren, wurde als Funktion des Abstands vom Hypozentrum berechnet

RÉSUMÉ

Les rapports de la dose absorbee par la thyroide et les gonades à la dose absorbee par les tissus dans l air ont ete determines en utilisant des sources de radiation simulees representant les rayons gamma et les neutrons initiaux émis par les bombes atomiques La dose absorbee dans ces organes critiques des survivants exposes aux rayons gamma et aux neutrons des bombes atomiques de 1945 a Hiroshima et à Nagasaki a eté estimee comme une fonction de la distance a l hypocentre

REFERENCES

AMAMOTO K BRILL A B FUJISAWA H HEYSEL R W, ITOGA T, NISHIMURA E and TOMONAGA M Leukemia in Nagasaki atomic bomb survivors ABCC Technical Report 11 59 Atomic Bomb Casualty Commission Hiroshima 1960

AUXIER J A CHEKA J S HAYWOOD F F, JONES T D and THORNGATE J H Free field radiation dose distributions from the Hiroshima and Nagasaki bombings Hlth Phys 12 (1966) 425

CHEKA J S Personal comunication (letter to ABCC) 1966

COGAN D G MATTN S F and KIMURA R J Atomic bomb cataracts Science 110 (1949), 659

HASHIZUME T MARUYAMA T SHIRAGAI A, TANAKA E, IZAWA M, KAWAMURA S and NAGAOKA S Estimation of the air dose from the atomic bombs in Hiroshima and Nagasaki Hlth Phys 13 (1967) 149

— — NISHIZAWA K and NISHIMURA A Dose estimation of human fetus exposed in utero to radiations from atomic bombs in Hiroshima and Nagasaki J radiat Res 14 (1973), 346

HIRAOKA T KAWASHIMA K INADA T and MATSUZAWA H Paired ionization chamber for measurement of neutrons and gamma rays Strahlentherapie 141 (1971), 64

— — and MATSUZAWA H Tissue-equivalent materials for neutron dosimetry, NIRS-report 2 National Institute of Radiological Sciences, Chiba 1973

[illegible]

MATSUZAWA H, INADA T HIRAOKA T HABU T and KAWASHIMA K Sensitivity of cavity ionization chamber for fast neutrons and gamma rays Nippon Acta radiol 29 (1969), 1030

NBS Handbook 75 (NCRP report No 25) Measurement of absorbed dose of neutrons and of mixtures of neutrons and gamma rays National Bureau of Standards, Washington 1969

of dose calculations for gamma-rays and protons from the $^{1}H(n,\gamma)D$ and $^{14}N(n,p)^{14}C$ reaction depends on systematic as well as on the statistical errors, namely the errors in the nuclear data used, estimated to be less than 6 per cent, the variations in the distance from the target to the counter, presumably less than 2 per cent, and the statistical error of 2 per cent in counting Thus the maximum error in the determinations of RATA for protons and gamma-rays from neutron reactions may be calculated to be 13 per cent, including errors of determinations of the in-air tissue absorbed dose with the paired chambers The total errors in the dose estimations for protons and gamma-rays were estimated to be about 20 per cent

Conclusion

The RATA $R(\Theta_0, \Phi_0)$ of an ovary ranged from a minimum value of 0 62 to a maximum value of 0 66 for the initial gamma-rays, 0 081 to 0 11 for recoil protons, 0 090 to 0 10 for gamma-rays from $^{1}H(n,\gamma)^{2}D$ reactions and 0 0050 to 0 0062 for protons from $^{14}N(n,p)^{14}C$ reaction, depending on a distance from the hypocenter and the posture of the survivors at the explosion time For testis, the RATA ranged from 0 67 to 0 88 for the initial gamma-rays, 0 30 to 0 60 for recoil protons, 0 013 to 0 025 for protons from the $^{14}N(n,p)^{14}C$ reaction and was 0 095 for gamma-rays from the $^{1}H(n,\gamma)^{2}D$ reaction

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CEREBRAL RADIATION SURGERY WITH NARROW GAMMA BEAMS

Physical experiments

BERT SARBY

Except some trials with 280 kV roentgen radiation (LEKSELL et coll 1955), the development of the therapeutic application of ionizing radiation for functional brain surgery has primarily utilized high-energy protons and other particles (LAWRENCE et coll 1962, LARSSON et coll 1963) The purpose of the present investigation was to provide an improved physical basis for attempts to use well-defined narrow gamma beams for cerebral radiation surgery It formed part of a systematic review of the conceivable radiation sources with regard to their physical properties, taking into consideration the clinical and economic requirements (LARSSON et coll 1974)

The principal conditions which must be fulfilled for application of electromagnetic or particle radiation to cerebral radiation surgery have been evaluated previously (LIDÉN 1957, LARSSON 1961, 1962) The measurements and calculations in the present investigation were based on gamma radiation from ^{60}Co since this (LARSSON et coll 1974) is the type of radiation most suitable at the present time Qualitatively, the results should also apply to other types of elec-

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RITCHIE R H and HURST G S Penetration of weapons radiation Application to the Hiroshima and Nagasaki studies Hlth Phys 1 (1959) 390

ROSSI H H Neutrons and mixed radiations *In* Radiation dosimetry p 667 Edited by G S Hine and G L Brownell Academic Press New York 1956

RUSSELL W L (a) Effect of the interval between irradiation and conception on mutation frequency in female mice Proc nat Acad Sci 64 (1965) 1552

— (b) Studies in mammalian radiation genetics Nucleonics 23 (1965) 53

SMITH J W and BOOT S J The variation of neutron dose with depth in a tissue equivalent phantom AERE R 3696 1961

TERANISHI E Personal communication 1966

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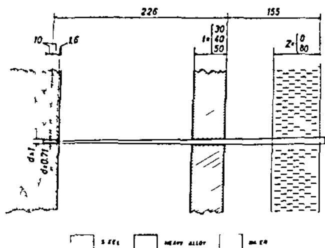


Fig 1 Idealized collimating system for estimating the distribution of the dose in narrow gamma beams The ^{60}Co source encapsulated in steel The horizontal and vertical scales are different (Dimensions in mm)

tromagnetic radiation in the energy range 0 4 to 10 MeV for which the Compton effect is the dominant type of interaction in soft tissue

The design of the beam geometry and the collimating systems for the individual channel is of decisive importance since it determines the energy flunce rate and the gradient of the cross-sectional distribution in the target volume It also affects the integral dose to the brain due to unwanted radiation

Theoretical considerations

Model for a beam channel

The essential geometrical requirements for multiple beam irradiation of small intracranial structures are given by LARSSON et coll (1974) The arrangement (Fig 1) provides a basis for discussing the design of the individual beam channel The target volume is assumed to be at a depth of Z = 8 0 cm in a water phantom simulating the head of a patient Because the radiation source has a finite lateral extension the collimator should be placed as close as possible to the irradiated object in order to limit the geometrical penumbra (LIDEN 1957) The collimator is positioned only 155 mm from the beam focus, i e the point of intersection of the beam axes, where the centre of the target volume is to be positioned Thus 125 mm is reserved for head fixtures and 30 mm for space occupied by necessary extra collimators (Fig 3) The radiation source is placed as close to the beam focus as the geometrical penumbra allows in the conceived application (Fig 1) This results in optimum use of the radiation source and also permits the dimensions of the radiation shield to be kept within practical limits The chosen distance between the centre of gravity of each radiation source and the beam focus is 38 cm

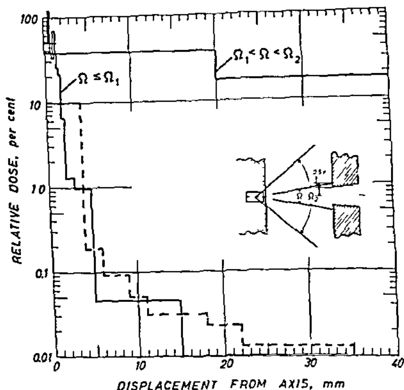


Fig 2 Mon ... to the be ... relative d ... ($\Omega_1 < \Omega$ ... immediate ... distribution of the absorbed dose from an ide ... tip of the cone defined by the aperture Th ... dose on the beam axis at Z = 10 mm

In the decay of ^{60}Co one 1 33 MeV and one 1 17 MeV photon are emitted per disintegration (in all the calculations 1 Ci ^{60}Co has been assumed to emit $2 \times 3.7 \times 10^{10}$ 1 25 MeV photons per second) The radiation source was a cylindrical cobalt rod (diameter 1 mm, length 20 mm) mounted in steel coaxially with a conical collimator (The reason for selecting d = 1 mm was that when the experiments were planned the only suitable active material commercially available was in the form of cylindrical pellets of this dimension) To simplify calculation the collimator was given a circular aperture so as to define a conical beam with a diameter of 2 5 mm at the target When extrapolated into

Table 1

Relative number of Compton electrons and their maximum ranges in water for varying scattering angles and corresponding recoil energies

Scattering angle u°	Relative number of electrons	Electron energy T (MeV)	Maximum range R (mm)	Lateral width of diffusion zone R sin u (mm)
0	1.000	1.05	4.55	0
15	0.410	0.90	3.77	0.98
30	0.166	0.62	2.32	1.16
45	0.091	0.35	1.04	0.74
60	0.018	0.145	0.263	0.23
75	0.022	0.036	0.0236	0.02
90	0	0	—	—

the rod, the cone describes a surface approximately coinciding with the inertial cylinder of the source. The material of the collimator is heavy alloy (density = 17.2 g/cm^3, HVL = 7 mm) of varying thickness. The ideal conditions to be strived for would permit the absorbed dose in water to be uniformly distributed within the geometrical edges of the beam and the dose at every point outside it to be negligible. The factors which make it difficult to achieve ideal conditions are the following:

1) *Finite source size.* The diameter of the cobalt rod, 1 mm, gives rise to (a) geometrical penumbra, (b) increased penetration of the radiation through the edges of the collimator (transmission penumbra), and (c) an increased influence of Compton scattering in the material of the collimator (wall effect) (STANTON 1959, GSCHEIDLEN et coll. 1960).

2) *Source enlargement.* Radiation emanating laterally from the cobalt source may be scattered in the surrounding steel so as to penetrate the aperture indirectly.

3) *Leakage radiation.* As the thickness of the collimator is reduced, untoward radiation can penetrate through the collimator material to an increasing extent.

4) *Unavoidable secondary radiation.* When the gamma radiation penetrates the object, Compton scattering gives rise to degraded photons outside the geometrical edges of the beam. The relative importance of this 'unavoidable' second-

ary radiation component increases with increasing depth of penetration for the object thicknesses of practical interest

5) *Electron diffusion* Diffusion of secondary electrons contributes to the absorbed dose outside the geometrical edges of the beam, and for a certain radiation quality, is also an unavoidable source of beam distortion Further, bremsstrahlung from the secondary electrons also gives rise to a small dose contribution in the whole irradiated volume

6) *Self-absorption in the source* The material of the source affects the energy spectrum of the photons and thereby also the entire dose distribution, partly through 'source enlargement' and partly through degradation of the gamma spectrum, in the primary beam

The ability to influence the dose distribution in an individual beam is limited, in practice, to modification of the collimator design The remaining parameters are more or less determined by the mentioned geometrical relationships or unavoidable dose contributions

Theoretical aspects of the design of an optimum collimating system

Of the factors listed above which have a negative influence on the geometrically ideal beam, only 1 (b), 1 (c) and (3) could be appreciably affected by the design of the collimator A starting point for analysing these effects was provided by a Monte Carlo computation (LEIMDORFER 1963) of the distribution of the absorbed dose in a plane perpendicular to the axis (at $Z = 80$) in water irradiated as illustrated in Fig 1 with $t = 30$ mm Fig 2 gives the relative transverse dose distribution perpendicular to the beam axis due to radiation which has penetrated the aperture of the collimator or its immediate vicinity in the solid angle $\Omega \leqslant \Omega_1$, and relates to it the leakage radiation within the relevant solid angle $\Omega_1 < \Omega < \Omega_2$ The effects of finite source size and source enlargement are included For comparison, the corresponding distribution for an ideal point source positioned at the tip of the cone defined by the collimator was calculated It seems that, within the limits of accuracy permitted by the relatively crude method of calculation, the effects of the finite extension of the source perpendicular to the beam axis are negligible

The large contribution to the relative dose within $\Omega_1 < \Omega < \Omega_2$ in Fig 2 is due to the small t value This leakage can be reduced to almost any desired extent by increasing t within the space available between the radiation source and the outermost edge of the collimator It is convenient, however, to achieve this reduction by introducing, instead, a primary collimator with the intention of diminishing, as far as possible, the radiation which leaks out in the solid angle

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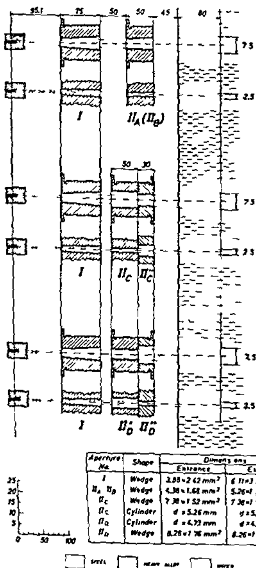
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Aperture No.	Shape	Dimensions	
		Entrance	Exit
I	Wedge	3.88×2.62 mm²	6.11×3.26 mm²
II_A, II_B	Wedge	4.38×1.68 mm²	5.26×1.92 mm²
II_C	Wedge	7.38×1.52 mm²	7.38×1.76 mm²
II_C	Cylinder	d = 5.26 mm	d = 5.26 mm
II_D	Cylinder	d = 4.73 mm	d = 4.93 mm
II_D	Wedge	8.26×1.76 mm²	8.26×1.92 mm²

Fig 3 The four collimator designs used in the measurements The horizontal and vertical scales are different. The ^{60}Co source has been drawn in position 1 (p 435) In position 2 the distance between the collimator and the centre of the source was instead 81 mm (Dimensions in mm)

$\Omega_1 < \Omega < \Omega_2$ In this way difficulties in manufacturing the collimating system are reduced and the clinically desired variation of the useful beam size can be brought about more simply and inexpensively In addition, a divided collimator seems to be able to reduce the significance of the wall effects as compared with the case of simple long collimators

Table 2

Symbols and numerical values for quantities used for dose calculations

Quantity	Symbol	Numerical value		Comments
		Position 1*	Position 2*	
Mass attenuation coefficient of water (cm²/g)	$\frac{\mu}{\varrho}$	0 063	0 063	At a photon energy of 1 25 MeV (cf ICRU 1959)
Mass energy absorption coefficient of water (cm²/g)	$\frac{\mu_{en}}{\varrho}$	0 030	0 030	— —
Density of water (g/cm³)	ϱ	1 0	1 0	
Conversion factor for MeV/g to rad		1 60 10 •	1 60 10 •	
Mean energy of the photons (MeV)	E_γ	1 25	1 25	
Number of photons per disintegration	n	2	2	
Nominal activity of the source (Ci)	A	22 8	21 6	Calculated from the specification of the manufacturer
Factor due to self absorption in the source	k_s	0 65	0 65	cf Fig 6
Factor due to attenuation in the material encapsulating the source	k_e	0 51	0 94	Corresponding to 15 9 mm and 1 6 mm steel in front of the source respectively
Distance to the centre of the source (cm)	R	39 51	38 10	cf Figs 1 and 3
Depth in the phantom (cm)	z	1 0 8 0	1 0 8 0	Reference depth Depth at beam focus
Dose rate (rad/s)	$D_{z=1}$	0 024	0 045	Calculated from equation (2)
Dose rate (rad/s)	$D_{z=8}$	0 010	0 019	Calculated from equation (2)

* For definition see p 435

Table 4

Measured dose rates at depths of 1 cm and 8 cm and the dose rate at a depth of 8 cm in relation to that at 1 cm (for definition of the positions see p 435)

Collimator	Position 1			Position 2		
	Dose rate 1 cm (rad/s)	Dose rate 8 cm (rad/s)	Relative dose rate (%)	Dose rate 1 cm (rad/s)	Dose rate 8 cm (rad/s)	Relative dose rate (%)
A	0.0223	0.0091	40.8	0.0520	0.0202	38.8
B	0.0272	0.0091	42.9	–	–	–
C	0.0210	0.0089	42.4	–	–	–
D	0.0212	0.0095	44.8		–	–
Mean value	0.0214	0.0092	42.7	0.0520	0.0202	38.8

As a result of the build up process, mentioned in point 4, the absorbed dose at large displacements from the axis (10 mm or more) increases by a factor of 2 to 3 at a depth of 8 cm as compared with 1 cm

The significance of the length of the radiation source The length of the radiation source was selected with regard to the desired dose rate at the target, taking into account the specific activity and cost of the source material The specific activity of commercially available ^{60}Co was between 150 and 200 Ci/g It is both clinically and technically desirable that the activity of each individual radiation source is so high that it delivers a dose rate of at least 1 rad/min at the beam focus (LARSSON et coll 1974) In order to determine the optimum length of the source the gain in dose rate was weighed against the relative loss of radiation due to self-absorption in the source The theoretical utilization factor k_s which denotes the fluence rate under the influence of self-absorption in a source of finite size relative to the fluence rate from a point source of corresponding activity can be expressed as

$$k_s = \frac{1 - e^{-\mu_s L}}{\mu_s L} \quad (1)$$

where L is the length and μ_s is the attenuation coefficient of the material of the source Fig 6 illustrates the utilization factor and the dose rate at a depth of 8 cm in water as a function of the length of the cobalt rod (calculated for a source diameter of 1 mm, $\mu_s = 0.47$ cm^{-1} and a specific activity of 150 Ci/g) Any extension of the source rod beyond 20 mm only contributes to a maximum of 35 per cent of what the corresponding increase in the activity of a point source

Table 3

Errors in the photographic dosimetry specified for a confidence level of 95 per cent

Source of error	Error
Exposure times	± 0 3 %
Calibration procedure (including the variations in the development procedure)	± 6 %
Densitometer reading	± 3 %
Overall uncertainty in relative dose determinations	± 7 %
Ionization chamber measurements	± 10 %
Overall uncertainty in absolute dose determinations	± 12 %

Electron diffusion It appears to be difficult to achieve experimental separation of the effect of diffusing secondary electrons However, the extent of the diffusion zone may be estimated from Table 1 giving the Klein-Nishina (NELMS 1953) electron distribution, for varying scattering angles u, the corresponding electron energies T and the corresponding maximum ranges R (BERGER & SELZER 1964) It was found that the lateral extent of the electron paths R sin u does not exceed 1 17 mm for u = 30° and that electrons produced at angles larger than 15° have a small relative contribution compared with recoil electrons produced in the forward direction Because of the multiple scattering only a fraction of the electrons reach R, a fact that further reduces the effective width of the diffusion zone to about 0 5 mm That the width of the zone is 0 5 mm or less can also be estimated from the data in Figs 7 and 8

Geometrical penumbra Any desired reduction of the geometrical penumbra width can be achieved by arbitrarily increasing the distance between source and collimator or by reducing the size of the radiation source Such measures are of little value when the effect of the penumbra is smaller than that of the unavoidable electron diffusion already discussed For the geometry in Fig 1 their contributions to the transverse dose distribution should be of comparable magnitude

Unavoidable secondary radiation Due to Compton collisions in the object, photons are scattered out of the geometrical beam Fig 9 gives the result of a Monte Carlo calculation of the dose distribution, due to this secondary radiation, in two planes perpendicular to the beam axis at two depths in water, ($z = 10$ mm and $z = 80$ mm) in accordance with Fig 1 (LEIMDORFER 1963)

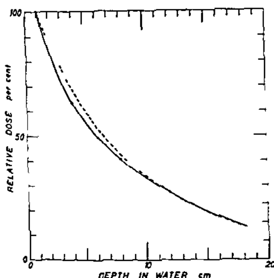


Fig 5 Experimental (solid line) central depth dose curve for the gamma beam in Fig 4 compared with the corresponding curve calculated from equation 2 for an ideal 1 25 MeV gamma beam with the same geometry (dashed line) The curves have been normalized to 100 % at a depth of 1 cm

mental arrangements are shown in Fig 3 A 23 Ci cobalt 60 source was used When the source with its surrounding capsule was delivered from the manufacturer it had been mounted the wrong way round (position 1) As a result there was a 15 9 mm layer of steel in front of the source (Fig 3), a mistake that was not discovered until a series of experiments had been performed This was later corrected and the source was placed in its correct position (position 2), with 1 6 mm of steel in front of it (Fig 1) The effects of the rearrangement were analysed and it appeared that the effect of the extra steel filtration on the dose distribution was practically negligible (Fig 11)

Design of experimental collimators

In accordance with the above guidelines for the design of an optimum beam defining system, four different divided collimating systems were constructed (Fig 3) All combinations were chosen to give, geometrically, a beam with a rectangular cross-section, 7 5 mm × 2 5 mm with a penumbra width of 0.5 mm, at the beam focus In collimator alternatives A, C and D the material was heavy alloy (90 % tungsten, 7 % nickel and 3 % copper, density 17 2 g/cm³) and in collimator B, which was identical in shape to A, it was steel (density 7 8 g/cm³)

Dosimetry

Dose distributions were measured with photographic film, Kodirex and a densitometer, Quantalog, with a circular measuring spot 0 1 mm in diameter

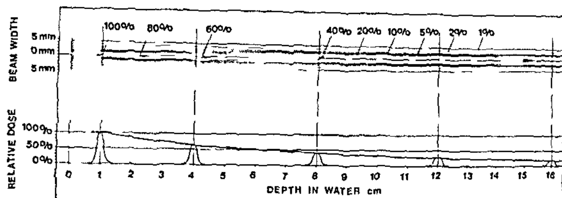


Fig 4 Isodose curves measured in a principal plane of a 2.5 mm wide ^{60}Co beam defined by collimator A and normalized to 100 % at a depth of 1 cm

would permit The length of 20 mm recommended was considered a suitable compromise

Calculation of the dose rate in a narrow gamma beam

The term narrow gamma beam implies here that the dose within the beam in an irradiated object originates from the first interaction of the photons and that the dose contributed by scattered photons is negligible The dose rate at a point in a narrow beam is thus proportional to the mass energy absorption coefficient of the target material and the energy fluence rate in the beam For the actual irradiation geometry (Figs 1, 3), the following relationship can be derived for the dose rate $\dot{D}(z)$ on the central axis of the beam in the water phantom

$$\dot{D}(z) = \frac{\mu_{en}}{\varrho} \cdot \frac{1.60 \cdot 10^{-6} \cdot E_\gamma \cdot 1 \cdot A \cdot 3.7 \cdot 10^{10} \cdot k_s \cdot k_c}{4\pi R^2} \cdot e^{-\mu z} \qquad (2)$$

The definition of the symbols used in equation 2 and the numerical values for the two different positions of the measurements are given in Table 2 The overall uncertainty of the dose rate values is 7 %, which is mainly ascribed to the uncertainty of the activity

Experimental methods

The experimental channel

It was considered suitable to test the properties of the collimators experimentally and an experimental channel was constructed so as to permit various types of divided collimators to be arranged easily The essential features of the experi-

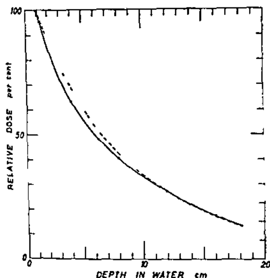


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Dosimetry

Dose distributions were measured with photographic film, Kodirex and a densitometer, Quantalog, with a circular measuring spot 0 1 mm in diameter

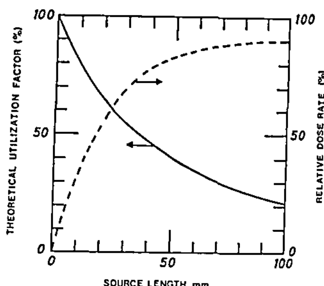
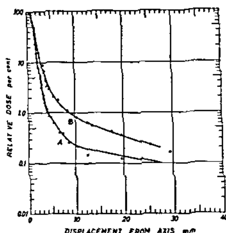


Fig 6 The theoretical utilization factor (solid line) and the relative dose rate at the beam focus (8 cm depth) (dashed line) as functions of the length of the cobalt rod (For a specific activity of 150 Ci/g the relative dose rate of 100 % corresponds to a dose rate of 2 rad/min)

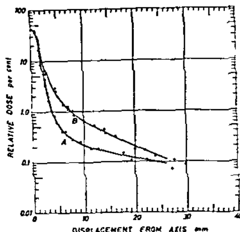
All films used for quantitative evaluation of relative doses were accompanied through a temperature-stabilized procedure of development by calibration films, which had been exposed to varying doses in a broad ^{60}Co gamma beam Determination of the dose absorbed in the calibration films was made by a Philips No 34780 ionization chamber with integrating electrometer In order to avoid the effects of statistical fluctuations in the distribution of silver grains (at low doses) and saturation (at high doses), two sets of films corresponding to exposure for 60 minutes and 2 minutes, respectively, were used for each collimator alternative For collimator C an additional exposure of 10 hours was employed

The film should have the same response for the absorbed dose independent of the spectral distribution at the measurement point, a condition that was easily fulfilled for measurements in any narrow beam of primary photons (MAUDERLI et coll 1960) Comparison between the theoretical and measured values of relative doses lends support to this assumption (Tables 2, 4) The uncertainty in the photographic dosimetry (summarized in Table 3) was estimated on the basis of the variations in the various phases of the procedure as recorded in repeated experiments

For determination of the dose outside the geometrical edges of the beam the condition above is not so well fulfilled, however, since the spectral distribution of the photons is affected by degradation (EHRLICH 1954, DUDLEY 1966) The maximum uncertainty due to these effects that is likely to occur in the outer portions of the beam was estimated not to exceed 30 per cent This estimation was made by means of measurements with the ionization chamber for collimator

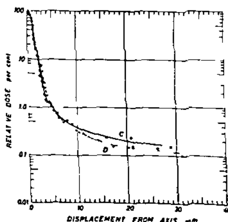


a

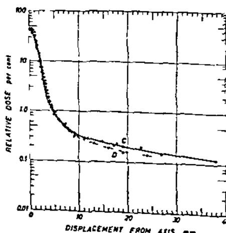


b

Fig 7 Transverse dose distributions for a depth of (a) 1 cm and (b) 8 cm in beams defined by the geometrically identical alternatives A (heavy alloy) and B (steel) The curves represent the distributions along the shorter axis of symmetry of the beam and they are normalized relative to the dose on the beam axis at a depth of 1 cm



a



b

Fig 8 Transverse dose distributions for a depth of (a) 1 cm and (b) 8 cm in beams defined by the collimator alternatives C and D The doses have been normalized relative to the dose on the beam axis at a depth of 1 cm

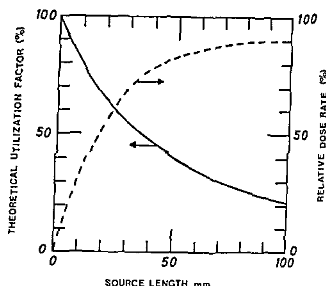
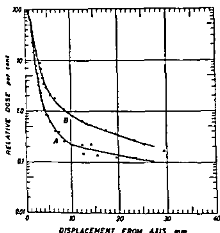


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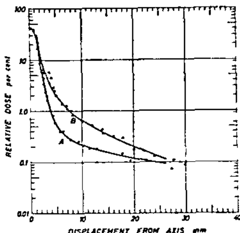
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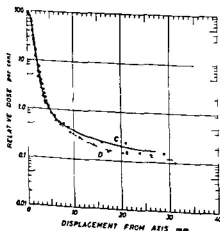


a

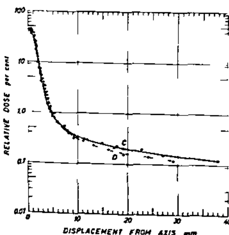


b

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a



b

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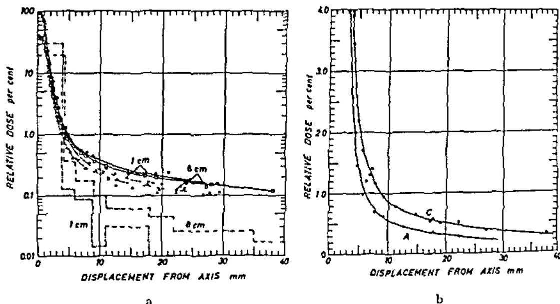


Fig 9 a) Transverse dose distributions for depths of 1 cm and 8 cm in a beam defined by collimator alternatives A and C The doses have been normalized relative to the dose on the beam axis at a depth of 1 cm For comparison, the results are given of a Monte Carlo calculation of the corresponding dose distributions in the neighbourhood of an ideal thin beam from a point source of the same apparent activity and collimated to a geometrical cross section of 7 5 mm × 2 5 mm at a depth of 8 cm - - ideal thin beam, ··· alternative A, — alternative C b) Transverse dose distributions at a depth of 8 cm drawn with linear scales and normalized to the dose on the beam axis at the same depth

alternative C at a depth of 8 cm in water and with displacements of 20 mm and 35 mm from the beam axis

Results

Dose distributions for various collimator alternatives (A—D)

The results are presented in the form of transverse distributions of absorbed dose perpendicular to the beam axis at different depths in the water phantom on the 'short' axis of symmetry of the beam cross-section For collimator A a complete distribution of isodose curves is also given for the corresponding principal plane (Fig 4) It appears that the central depth dose curve in a 2 5 mm × 7 5 mm ^{60}Co beam well conforms with the exponential, theoretical depth-dose curve for a narrow 1 25 MeV photon beam of corresponding geometry, calculated according to equation 2 (Fig 5) Transverse dose distributions for collimator alternatives A and B for depths of 1 cm and 8 cm are given in Fig 7 and corresponding distributions for collimators C and D in Fig 8 Rearrange-

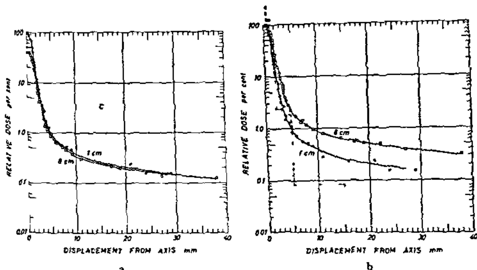


Fig 10 a) Transverse dose distributions at depths of 1 cm and 8 cm in a beam defined by alternative C The doses are normalized to the dose on the beam axis at a depth of 1 cm b) The same dose distributions individually normalized to 100 % on the beam axis The corresponding theoretically calculated dose distribution for a depth of 8 cm is also given (from Fig 2 and normalized to a depth of 8 cm) theoretical case — alternative C

ment of the source from position 1 to position 2 had little effect (Fig 11), a fact that illustrates implicitly that the length of the source has little importance for the dose distribution

The transverse dose distribution for a depth of 8 cm in a 2 5 mm wide 185 MeV proton beam and the corresponding depth dose curves are given in Figs 12 and 13, together with the theoretical and experimental dose distributions for a narrow gamma beam From comparison of the curves it seems likely that gamma radiation from ^{60}Co in beams of the dimensions considered could be used to produce the same biologic effect in the region for the central acute radiation lesion as observed after irradiation with protons

Determination of the dose rate on the beam axis

The dose rates obtained on the beam axis at depths of 1 cm and 8 cm in water for the various collimator alternatives appear in Table 4 The measured dose rates and relative doses show good agreement with corresponding theoretical values (Table 2) within the limits of their uncertainties (Table 3) The theoretical depth dose curve (Fig 5) was therefore also considered applicable to all the collimator alternatives

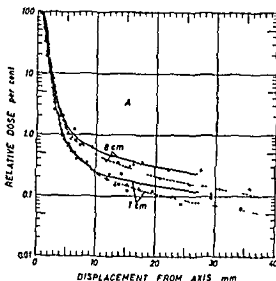


Fig 11

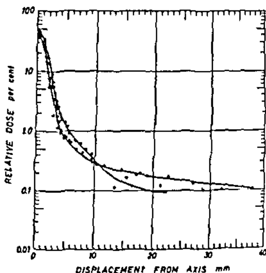


Fig 12

Fig 11 Transverse dose distributions at depths of 1 cm and 8 cm in beams defined by alternative A with the source the 'wrong' way round (— position 1) and the 'right' way round (position 2) In each case the distributions have been normalized to a 100 % dose on the beam axis at the respective depths This figure also illustrates the minor importance of the length of the source and source enlargement for the dose distribution

Fig 12 Comparison between transverse dose distributions at a depth of 8 cm in a ^{60}Co beam collimated by alternative C and a rectangular parallel 185 MeV proton beam collimated by a plane parallel 2 5 mm wide slit in 50 mm brass (LARSSON & SARBY 1974) ○○ protons experiment II ●● ^{60}Co aperture C

Discussion

The calculations indicate that the demand on the dose rate in the single beam can be fulfilled The clinical usefulness of gamma radiation for cerebral radiation surgery is supported by the comparison of the data (Figs 12, 13) with dose distributions for 185 MeV proton radiation which has been used previously in clinical application (LARSSON et coll 1963, LARSSON & SARBY 1974) The transverse dose distributions for a depth of 8 cm are very similar for displacements of up to 10 mm from the beam axis, while at greater displacements the ^{60}Co dose exceeds the proton dose by approximately 100 per cent The distributions along the axes for proton and ^{60}Co beams are also similar for those depths at which planned lesions normally lie, 6 to 10 cm (Fig 13)

The physical factors determining the energy fluence rate in the target volume are the specific activity of the source material and transmission and scattering in source, collimators and brain The evaluation of alternative source-collimator systems is also highly dependent on limitations imposed by material and manufacturing costs Shaping of well-defined, narrow gamma beams for clinical use

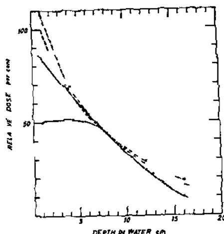


Fig 13 Central depth dose curves for the same ^{60}Co beam and proton beam as in Fig 12 compared with the theoretical depth dose curve (from Fig 5) All the curves have been normalized relative to the same value at a depth of 8 cm Experiment I with protons was performed with a secondary radiation collimator and experiment II without such a collimator The object of this collimator was to eliminate protons scattered in the beam-defining collimator ——— protons experiment I —O—O—O protons experiment II --x--x-- ^{60}Co experimental ^{60}Co theoretical

with uniform, high energy fluence rates thus requires a careful compromise between geometrical and physical relationships and practical considerations

The design of the beam geometry (Figs 1, 3) was based on the assumption that geometrical penumbra effects and diffusion of secondary electrons influence the dose gradient in the border zone of the target volume in a given way The differences between the cases may thus be mainly ascribed to differences in transmission and scattering effects in the collimators

Choice of collimator geometry

Comparison between the transverse dose distributions in Figs 7, 8 and 9 shows that a beam shaped by collimator A provides the most favourable gradient in the penumbra region as well as the lowest integral dose to surrounding brain tissue The suitability of using steel as collimating material, which would be more easily machined and less expensive, is disproved by Fig 7 Such an arrangement would increase the radiation energy absorbed outside the primary beam by a factor of 2 to 3 This would be unacceptable at the dose levels in question for the planned applications (LARSSON *et coll* 1974)

It was considered that great practical and economic advantages would be gained if variation of the size of the beam cross section could be facilitated This was achieved by dividing the beam-defining collimator II into two parts as illustrated in Fig 3 in two alternative designs C and D For C the part of the collimator defining the 7 5 mm diameter of the beam cross section, the dimension most likely to be varied, is placed in the most accessible position, i e nearest

the head of the patient, but for D it is placed between the other two parts of the collimating system. Alternative C, which has practical advantages, does not appear to be significantly inferior to D (Fig. 8). A comparison of alternatives C and A (Fig. 9) demonstrates that irradiation with a beam defined by C would give an approximately 50 per cent higher dose in the region nearest to the planned radiation lesion, due to wall effects in its beam-defining collimators. In spite of the desirability of reducing the dose in this region as much as possible, alternative C was nevertheless preferred because of its practical and economic advantages.

The properties of collimator C can further be evaluated from Fig. 10. The dose falls rapidly in the vicinity of the geometrical edge of the beam, and at displacements from the axis of 5 mm and 9 mm at depths of 1 cm and 8 cm respectively it has dropped to approximately 1 per cent of the dose at the beam axis. Comparison between the experimental and the theoretically calculated distributions for the idealized collimating arrangement (Fig. 1) shows good agreement within the geometrical beam and the penumbra region. It may be concluded that further attempts to reduce transmission penumbra effects by modifying the collimator design would only give insignificant improvement of the dose gradient. Outside the geometrical penumbra region collimator C (and A) provides a somewhat less well defined situation than would appear to be theoretically possible. Fig. 9 shows the transverse distributions for collimators A and C compared with corresponding calculated dose distributions for an ideal 1.25 MeV gamma beam. At displacements exceeding 4 mm from the beam axis the unavoidable secondary radiation dose increases 2- to 3-fold when the depth is increased from 1 cm to 8 cm. The difference between the experimental and the theoretical curves, i.e. the dose due to wall effects, leakage radiation and source enlargement, resulting from imperfections in the collimating arrangement, amounts to roughly 80 per cent of the total dose. The dose due to leakage radiation of undegraded photons from the source is in the order of 10^{-4} % (HVL = 7 mm). The contribution from source enlargement is small, as mentioned above (Fig. 2). Indirectly, an idea of its magnitude can also be obtained from Fig. 11.

Theoretically, further improvement of collimation would be possible. Such improvement is desirable in order to reduce the integral dose to the brain. In particular, it should be possible to reduce the contribution from wall effects in the primary collimator with the object of reducing leakage radiation through the collimating material. A possible modification would be to move this collimator and change its shape and its entry opening so that no part of the wall of the collimator is visible from the region surrounding the beam focus. However, in view of the limited space available no essential improvement can be expected.

Electron radiation from the beam channel

The gamma beam is contaminated with electron radiation originating from Compton collisions in the beam channel The patient's skin could receive an appreciable dose contribution in this way In order to investigate this risk, rabbits' ears were irradiated in the beam for periods of 10 hours, i e with six times the dose to be used in the planned treatments (LARSSON et coll 1974) During seven months of observation no macroscopic changes were observed and it is probable that the contribution of electron radiation can be ignored in clinical applications

Practical conclusions

The experiments and calculations reported here have been useful in the development of a clinically useful apparatus for cerebral radiation surgery (LARSSON et coll 1974) The findings may be summarized as follows

1) The physical conditions for the use of narrow gamma beams for cerebral radiation surgery can be considered favourable with regard to fluence rate and collimation This conclusion is based on the similarity of the dose distributions to the proton dose distributions which have previously been used for the same purpose (Figs 12, 13)

2) In a clinical apparatus for radiation surgery using ^{60}Co or other suitable kinds of gamma radiation (LARSSON et coll 1974) a divided beam-defining collimator corresponding to alternative C (Fig 3) would be the most appropriate because of its practical and economic advantages (For a linear accelerator or other high energy roentgen radiation source with one beam channel a collimator of type A might nevertheless be preferable)

3) Unwanted dose contributions outside the geometrical edges of the beam due to scattering and transmission processes in the proposed ^{60}Co channel can mainly be attributed to wall effects in the collimators To a certain degree these effects could be reduced by means of a modification of the primary collimator without affecting its main purpose of limiting leakage radiation

Acknowledgement

This investigation was supported by the Swedish Medical Research Council

SUMMARY

The physical and geometrical demands on the single beam channel for the precise irradiation of small intracranial structures with ^{60}Co gamma radiation were evaluated Model experiments performed with the aid of photographic dosimetry on various types of collimating systems in combination with theoretical calculations on an idealized beam geometry showed the possibility of shaping well-collimated gamma beams with a sufficiently

high energy fluence rate. Unwanted contributions to the dose in regions outside the geometrical edges of the primary beams due to scattering and transmission effects were analysed.

ZUSAMMENFASSUNG

Die physikalischen und geometrischen Forderungen an dem einzelnen Strahlenkanal zur genauen Bestrahlung kleiner intrakranialer Strukturen mit ^{60}Co Gamma-Bestrahlung wurden festgestellt. Modellexperimente, die mit Hilfe der photographischen Dosimetrie an verschiedenen Typen von kollimierenden Systemen vorgenommen wurden, zeigten in *Kombination mit theoretischen Berechnungen an einer idealisierten Strahlengeometrie* die Möglichkeit, gut-kollimierte Gamma-Strahlenverläufe mit genugend hohen Energie-Fluss-Geschwindigkeiten abzugrenzen. Unerwünschte Beiträge zur Dosis in Gebieten ausserhalb des geometrischen Fokus der Primärstrahlung infolge von Streuung und Transmissionseffekten wurden analysiert.

RÉSUMÉ

L'auteur a étudié les conditions physiques et géométriques imposées à chaque canal de faisceau pour l'irradiation précise de petites structures intracrâniennes par le rayonnement gamma du ^{60}Co. Des expérimentations sur modèle faites au moyen de la dosimétrie photographique sur différents types de système de collimation associées à des calculs théoriques sur une géométrie de faisceau idéalisé ont montré la possibilité de former des faisceaux gamma bien collimatés avec un flux d'énergie suffisamment haut. L'auteur a étudié l'irradiation indésirable dans des régions situées hors des limites géométriques des faisceaux primaires due aux effets de dispersion et de transmission.

REFERENCES

Ber[illegible]

Dudley R [illegible] Dosimetry with [illegible] p. 325. Edited by F. H. Attix and W. C. Roesch, Academic Press, New York and London 1966.

Ehrlich M. Photographic dosimetry of x- and gamma rays. U.S. National Bureau of Standards Handbook 57 (1954).

Gscheidlen W., Malsch F. und Schittenhelm R. Der Halbschatten bei der Kobalt-Fernbestrahlung. Strahlentherapie 111 (1960), 621.

ICRU. Report of International Commission on Radiological Units and Measurements. U.S. National Bureau of Standards Handbook 78 (1959).

Larsson B. Pre-therapeutic physical experiments with high energy protons. Brit. J. Radiol. 34 (1961), 143.

— On the application of a 185 MeV proton beam to experimental cancer therapy and neurosurgery. A biophysical study. Acta Universitatis Upsaliensis. Abstract of Uppsala dissertations in Science. No. 9 (1962).

— Leksell L. and Rexed B. The use of high energy protons for cerebral surgery in man. Acta chir. scand. 125 (1963), 1.

— LIDÉN K and SARBY B Techniques for irradiation of small structures through the intact skull To be published in Acta radiol Ther Phys Biol
— and SARBY B Equipment for radiation surgery using narrow 185 MeV proton beams Dosimetry and design To be published in a Suppl to Acta radiol
LAWRENCE J H, TOBIAS C. A, BORN J L, WANG C and LINFOOT J A Heavy particle irradiation in neoplastic and neurologic disease J Neurosurg 19 (1962), 717
LEIMDORFER M Personal communication (1963)
LEKSELL L HERNER T and LIDEN K Stereotaxic radiosurgery of the brain Report of a case Kungl fysiograf sallsk Lund förh 25 (1955), 142
LIDÉN K Physikalische Grundlagen fur die Verwendung ionisierender Strahlung bei gezielter Hirnchirurgie *In* Handbuch der Neurochirurgie, Vol 6, p 199 Edited by H Olivecrona and W Tonnis Springer, Berlin 1957
MAUDERLI W, GOULD D and LANE J Film dosimetry of cobalt 60 radiation Amer J Roentgenol 83 (1960), 520
NELMS A Graphs of the Compton energy angle relationship and the Klein Nishina Formula from 10 keV to 500 MeV National Bureau of Standards Circular 542, Washington 1953
STANTON L Penumbra measurements of supervoltage and cobalt 60 machines by a photographic method Radiology 73 (1959) 253

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CARCINOMA OF THE LARYNX

III. Therapeutic results

KARSTEN JØRGENSEN

During the period August 1963 to August 1968 152 patients with carcinoma of the larynx were treated, primary surgery was used in 5 cases and in 147 cases primary irradiation was administered by a ^{60}Co apparatus. The material has previously been presented (JØRGENSEN & SELL 1971) with calculation of the 5-year result, comparison with the results obtained during the preceding period with conventional radiation therapy, and assessment of the possible causes of recurrence after irradiation. The 5-year follow-up results were available for all patients in August 1973, and the present report will mainly describe the therapeutic results.

The incidence, sex ratio, age distribution, histology, symptoms and signs, classification, method of treatment, and follow-up were presented previously (JØRGENSEN & SELL). Only the classification will be briefly discussed in the present report.

Two closely related systems of classification existed before 1972. The American system, American Joint Committee (AJC), and the international system, Union Internationale Contre le Cancer (UICC) but in 1972 common rules for the TNM classification as well as staging were established (AJC 1972, UICC

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Table 1

Location of tumours in 152 cases of laryngeal carcinoma grouped according to the TNM system

Site	N0	N1a	N1b	N2a	N2b	N3
Supraglottic (41 cases)						
T1a	5					
T1b	5	1				
T2	9		1		1	
T3	2		3	1	1	
T4	7		2		1	2
Glottic (102 cases)						
TIS	8					
T1a	33					
T1b	11					
T2	25					
T3	19		1			
T4	3		1			1
Subglottic (9 cases)						
T2	1					
T3	1		1			
T4	4				1	1

1972) The cases included in the present series were re-classified and staged according to these rules (Tables 1, 2) On admission 11 per cent (17/152) were presumed to have regional lymph node metastases supraglottic cases 27 per cent (11/41), glottic 3 per cent (3/102) and subglottic 3 cases of 9 It is recommended, by AJC as well as by UICC, to aim at an assessment of the site of origin in each individual case, which has given the result presented in Table 3 In the more extensive cases, this assessment is rather uncertain, however

Information concerning smoking habits was available in only 46 per cent of the cases, this series thus gives no information on the possible aetiologic role of smoking The occupation was known in 88 per cent (133/152) but no particularly exposed groups were revealed

Results

The result of the cumulative calculations performed in the autumn of 1969 is presented in Fig 1 a In calculating the mortality coefficients of the individual

Table 2

Staging of the material

Stage	No of cases	Per cent
TIS	8	5 9
I	55	36 1
II	35	23 2
III	50	32 9
IV	4	2 6
Total	152	100

follow-up years, the expected 5-year survival was made out as being between 65 and 70 per cent The definitive result is illustrated by the curve in Fig 1 b The crude 5-year survival proved to be 68 per cent, and twice the standard error 7 6 per cent is marked on both sides of the curve A supplementary correction for deaths from causes other than laryngeal carcinoma was carried out by calculating the corrected mortality coefficients for the separate years The latter were calculated on the basis of the formula $q_n = \frac{c}{i/2+c+l}$ (NOHRMAN 1953, JØRGENSEN 1970), where q_n is the corrected mortality coefficient in the n'th year, c the number of deaths of the disease, i the number of deaths of other diseases, and l the number of patients alive at the end of the year concerned Most deaths due to carcinoma occurred within the first 2 years (Fig 1 b) Seventy-six per cent of all the patients must be assumed to have been cured of their laryngeal tumour

The therapeutic results in relation to stage and method of treatment are listed in Table 4 without regard to therapeutic procedures directed at regional lymph node metastases The majority of patients (103) received irradiation as the only treatment Primary total laryngectomy was the only treatment in 5 cases A residual tumour or recurrence following primary radiation therapy led to supplementary surgery 10 partial and 34 total laryngectomy The frequency of total laryngectomy among those who survived for more than 5 years appears in the table Out of the 104 with more than 5-year survival only 20 per cent (21/104) had lost their laryngeal function through total laryngectomy The frequency of total laryngectomy in the entire series was 26 per cent (39/152) The survival curves for stages I, II and III appear in Fig 2

Glottic group Table 5 includes the results of treatment of recurrences in the regional lymph nodes It is apparent that the prognosis for TIS and T1 cases generally is good but 8 recurrences occurred among 52 small vocal cord carcino-

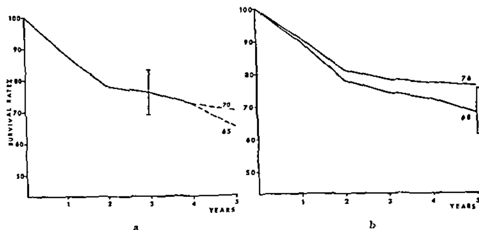


Fig 1 a) Cumulative survival curve for 152 patients with carcinoma of the larynx, calculated in 1969 b) Definitive survival The upper curve is corrected for mortality from other causes, the lower curve is uncorrected

mas Also in the T2 and T3 groups good results were obtained The survival was 94 per cent (49/52) in stage I, 72 per cent (18/25) in stage II, 63 per cent (15/24) in stage III and 0 per cent (0/1) in stage IV

The survival curves for the TJS + T1, T2 and T3 cases are given in Fig 3 The curve for the former group is corrected for deaths due to other diseases than

Table 3

Site of origin

Site		No of cases
Supraglottis		
Epilarynx	Posterior surface of suprahyoid epiglottis (including the tip)	12
	Aryepiglottic fold	9
	Arytenoid	1
Infrahyoid epiglottis		6
Ventricular bands		11
Ventricular cavities		2
Glottis		
Vocal cords		100
Anterior commissure		2
Subglottis		9

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Total	152	100

follow-up years, the expected 5-year survival was made out as being between 65 and 70 per cent The definitive result is illustrated by the curve in Fig 1 b The crude 5-year survival proved to be 68 per cent, and twice the standard error 7 6 per cent is marked on both sides of the curve A supplementary correction for deaths from causes other than laryngeal carcinoma was carried out by calculating the corrected mortality coefficients for the separate years The latter were calculated on the basis of the formula $q_n = \frac{c}{i/2+c+l}$ (NORRMAN 1953, JORGENSEN 1970), where q_n is the corrected mortality coefficient in the n'th year, c the number of deaths of the disease, i the number of deaths of other diseases, and l the number of patients alive at the end of the year concerned Most deaths due to carcinoma occurred within the first 2 years (Fig 1 b) Seventy-six per cent of all the patients must be assumed to have been cured of their laryngeal tumour

The therapeutic results in relation to stage and method of treatment are listed in Table 4 without regard to therapeutic procedures directed at regional lymph node metastases The majority of patients (103) received irradiation as the only treatment Primary total laryngectomy was the only treatment in 5 cases A residual tumour or recurrence following primary radiation therapy led to supplementary surgery 10 partial and 34 total laryngectomy The frequency of total laryngectomy among those who survived for more than 5 years appears in the table Out of the 104 with more than 5-year survival only 20 per cent (21/104) had lost their laryngeal function through total laryngectomy The frequency of total laryngectomy in the entire series was 26 per cent (39/152) The survival curves for stages I II and III appear in Fig 2

Glottic group Table 5 includes the results of treatment of recurrences in the regional lymph nodes It is apparent that the prognosis for TIS and T1 cases generally is good but 8 recurrences occurred among 52 small vocal cord carcino-

Table 6

Results of treatment of supraglottic and subglottic carcinoma

	No of cases	5-year survival				Crude 5-year survival rate
		Irradiation alone	Irr + surg for recurrences and residual tumours	Primary laryngectomy	Total	
Supraglottic						
T1	11	5/8	0/3	0	5/11	
T2	11	3/4	3/7	0	6/11	
T3	7	1/3	1/4	0	2/7	
T4	12	3/7	2/5	0	5/12	
Total	41	12/22	6/19	0	18/41	44 %
Subglottic						
T2	1					
T3	2	0/4	2/2	2/3	4/9	
T4	6					

carcinoma of the larynx, i e 98 per cent cured One patient died in the first year after treatment with metastases to the mediastinal lymph nodes but without signs of recurrence locally or in the cervical lymph nodes The patient was classified as having died of laryngeal carcinoma

Supra and subglottic groups The therapeutic results in these two groups (Table 6) are poorer as compared with the glottic cases The frequency of primarily N1b—N2b and N3b cases in the supraglottic group was 27 per cent (11/41) Moreover, regional lymph nodes were secondarily involved in 8 cases, which means that metastases to the regional lymph nodes occurred in 46 per cent (19/41) of the supraglottic group

Table 6 presents the results of the 41 supraglottic cases subdivided into T groups If the stages also are considered the following results appear stages I + II 55 per cent (11/20), stages III + IV 33 per cent (7/21) crude 5-year survival

Local residual tumours and recurrences During the 5-year observation period 57 cases of local residual tumours and recurrences occurred following primary radiation therapy and one case following primary laryngectomy The local re-

Table 4

Results of treatment in the total material

	Stage					Total
	TIS	I	II	III	IV	
5 year survival						
Number of cases	8	55	35	50	4	152
Irradiation alone	6/6	40/46	16/21	12/28	0/2	74/103
Primary laryngectomy	—	—	—	4/4	0/1	4/5
Irradiation + secondary partial laryngectomy	1/1	4/4*	3/4	1/1*	—	9/10
Irradiation + secondary partial laryngectomy, later total	—	0/1	1/1	—	—	1/2
Irradiation + secondary laryngectomy	1/1	2/4**	5/9	8/17***	0/1	16/32
Total	8/8	46/55	25/35	25/50	0/4	104/152
Crude 5 year survival rate	100 %	84 %	71 %	50 %	0/4	68 %
Corrected 5 year survival rate	100 %	89 %	82 %	59 %	0 %	76 %
Frequency of laryngectomized	1/8	3/47	6/25	12/25	—	21/104
cases at 5 years	13 %	6 %	24 %	48 %	—	20 %

* 1 cordectomy in each group

** 2 patients alive with lymph node recurrence at 5 years

*** 1 patient alive with local recurrence at 5 years

Table 5

Results of treatment of glottic carcinoma

	No of cases	5 year survival				Crude 5 year survival rates
		Irradiation alone	Irr + surg for recurrences and residual tumours	Primary laryngectomy	Total	
TIS	8	6/6	2/2	0	8/8	} 94 %
T1a	33	27/30	3/3	0	30/33	}
T1b	11	8/8	3/3	0	11/11	}
T2	25	11/14	7/11	0	18/25	72 %
T3	20	8/12	4/7	1/1	13 20	65 %
T4	5	1/2	0/2	1/1	2/5	
Total	102	61/72	19/28	2/2	82/102	80 %

Table 6

Results of treatment of supraglottic and subglottic carcinoma

	No of cases	5-year survival				Crude 5-year survival rate
		Irradiation alone	Irr + surg for recurrences and residual tumours	Primary laryngectomy	Total	
Supraglottic						
T1	11	5/8	0/3	0	5/11	
T2	11	3/4	3/7	0	6/11	
T3	7	1/3	1/4	0	2/7	
T4	12	3/7	2/5	0	5/12	
Total	41	12/22	6/19	0	18/41	44 %
Subglottic						
T2	1					
T3	2	0/4	2/2	2/3	4/9	
T4	6					

carcinoma of the larynx, i e 98 per cent cured One patient died in the first year after treatment with metastases to the mediastinal lymph nodes but without signs of recurrence locally or in the cervical lymph nodes The patient was classified as having died of laryngeal carcinoma

Supra and subglottic groups The therapeutic results in these two groups (Table 6) are poorer as compared with the glottic cases The frequency of primarily N1b—N2b and N3b cases in the *supraglottic group* was 27 per cent (11/41) Moreover, regional lymph nodes were secondarily involved in 8 cases, which means that metastases to the regional lymph nodes occurred in 46 per cent (19/41) of the supraglottic group

Table 6 presents the results of the 41 supraglottic cases subdivided into T groups If the stages also are considered the following results appear stages I + II 55 per cent (11/20), stages III + IV 33 per cent (7/21) crude 5-year survival

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Table 4

Results of treatment in the total material

	Stage TIS	I	II	III	IV	Total
5 year survival						
Number of cases	8	55	35	50	4	152
Irradiation alone	6/6	40/46	16/21	12/28	0/2	74/103
Primary laryngectomy	—	—	—	4/4	0/1	4/5
Irradiation + secondary partial laryngectomy	1/1	4/4*	3/4	1/1*	—	9/10
Irradiation + secondary partial laryngectomy, later total	—	0/1	1/1	—	—	1/2
Irradiation + secondary laryngectomy	1/1	2/4**	5/9	8/17***	0/1	16/32
Total	8/8	46/55	25/35	25/50	0/4	104/152
Crude 5 year survival rate	100 %	84 %	71 %	50 %	0/4	68 %
Corrected 5 year survival rate	100 %	89 %	82 %	59 %	0 %	76 %
Frequency of laryngectomized cases at 5 years	1/8 13 %	3/47 6 %	6/25 24 %	12/25 48 %	—	21/104 20 %

* 1 cordectomy in each group
** 2 patients alive with lymph node recurrence at 5 years
*** 1 patient alive with local recurrence at 5 years

Table 5

Results of treatment of glottic carcinoma

	No of cases	5 year survival: Irradiation alone	Irr + surg for recurrences and residual tumours	Primary laryngectomy	Total	Crude 5 year survival rates
TIS	8	6/6	2/2	0	8/8	
T1a	33	27/30	3/3	0	30/33	94 % (TIS, T1a, T1b)
T1b	11	8/8	3/3	0	11/11	
T2	25	11/14	7/11	0	18/25	72 %
T3	20	8/12	4/7	1/1	13/20	65 %
T4	5	1/2	0/2	1/1	2/5	
Total	102	61/72	19/28	2/2	82/102	80 %

Table 7

Treatment of residual tumours or recurrences

Complete laryngectomy	32
Partial laryngectomy	8
Partial laryngectomy, later complete	2
Endoscopic cordectomy	1
Cytostatic treatment	1
Palliative irradiation	2
No treatment*	12
Total	58

* Due to advanced age, poor general condition, or inoperability

through the thyroid cartilage and vocal cord on the unaffected side and the tumour resected The posterior part of, or in certain cases the whole, arytenoid cartilage was removed Superiorly the ventricle and false cord were also resected If the subglottis was involved by the carcinoma, the resection included the anterior part of the cricoid cartilage, with the mucosa The radicality was secured by microscopy of frozen sections in the course of the operation The partial laryngectomy was used in cases with small recurrences In several cases the type of operation, partial or total laryngectomy, was decided when the extent of the tumour had been determined at an exploratory laryngofissure

Total laryngectomies were usually performed through a V-shaped incision on the anterior aspect of the neck After tracheotomy the larynx was isolated and removed The hyoid bone with the pretracheal muscles was resected in cases of supraglottic tumour and if the prelaryngeal space was involved Supplementary resections of the base of the tongue and the hypopharyngeal mucosa were carried out in some cases The regional lymph nodes were always explored Microscopy of frozen sections of possibly metastatic nodes was performed and if metastases were found neck dissection was carried out In two cases recurrences were treated by palliative irradiation, with regression of the tumour and a favourable effect upon pain Methotrexate was administered in one case without evident effect

Secondary recurrences Repeat recurrence following partial laryngectomy of the first recurrence was treated in 2 cases by total laryngectomy One of these patients remained free of recurrence for more than 5 years, whereas the other one died of a local recurrence

Recurrences following total laryngectomy were usually beyond recovery but in one case irradiation was curative This patient had originally received ^{60}Co irra-

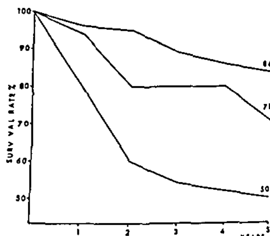


Fig 2 Survival curves for stages I (upper), II (middle) and III (lower) (uncorrected)

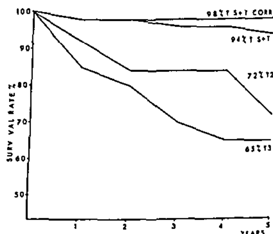


Fig 3 Survival curves for patients with carcinoma of the glottis The uppermost curve is corrected for mortality from causes other than carcinoma of the larynx All other curves are uncorrected

currences were 41 and their appearance time is given in Fig 4 Only 5 of the recurrences occurred later than the second year following treatment Seventeen residual tumours were encountered The method of treatment and the results in these 58 cases are given in Tables 4, 5 and 6 It must be mentioned that only one cordectomy is listed in Table 7, although Table 4 included 2 This is because no malignant tissue could be found at microscopy of one of these cases Total laryngectomy was performed in one case due to probable recurrence respiratory obstruction because of oedema and severe pain However, microscopy could only demonstrate a large necrotic area without evidence of malignant tissue This case is nevertheless included in all the tables as a recurrence

The partial laryngectomies were performed through a midline incision After the perichondrium had been dissected free, the larynx was incised vertically

Table 8

Results of treatment of regional lymph node metastases

	Primary	Secondary	All cases
5 year survival			
Number of cases	18	15	33
Irradiation curative	6/8*	1/1**	7/9
Irradiation palliative	0/1	0/3	0/4
Irradiation + neck dissection	1/6		1/6
Neck dissection	2/3	3/8**	5/11
No treatment		0/3	0/3
Total	9/18	4/15	13/33
Crude 5 year survival rate***	50%	27%	39%

* 1 patient alive with recurrence of local tumour
** 1 patient alive with inoperable regional lymph node metastases
*** Calculated from the first day of treatment of the primary local tumour

metastases in the regional lymph nodes Late nodal involvement occurred in 15 cases, all within the first 4 years after treatment (Fig 5) Thus, 33 patients, or 22 per cent, had verified or presumed lymph node metastases The frequency was highest among the supraglottic tumours, with 11 primary and 8 late metastases, i e 46 per cent (19/41) The metastases were located in the jugulo-digastric nodes in 19 cases, in 4 in the jugulo-omohyoid nodes, and in 7 cases in lymph nodes between these two groups Only in one case were both groups involved One patient had a prelaryngeal metastasis and also metastases in the contralateral supraclavicular region, and one only in the mediastinum This was the mentioned T1 NO MO glottic case but co-existing malignant disease could not be excluded with certainty Twenty five patients had involvement of homolateral lymph nodes, 6 bilateral and 2 contralateral involvement (one of these latter patients was the one with a prelaryngeal metastasis)

Primary full dose irradiation was used in 14 cases (Table 8) Of these 6 survived for more than 5 years, 8 developed recurrence in the irradiated area, 2 were inoperable Six underwent operation including neck dissection, one survived for more than 5 years, one died of distant metastases without recurrence in the neck and 4 of recurrence in the neck Table 8 also reveals that in 3 patients primary neck dissection was carried out, which proved curative in 2

Late metastases occurred in 15 cases, in 3 of these within the field irradiated at the treatment of the primary tumour and in 12 outside this field Eight under-

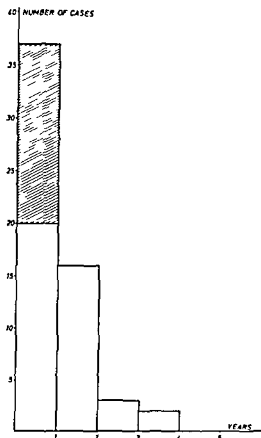


Fig. 4. Time of occurrence of recurrences in 41 cases. □ recurrences ▨ residual tumour

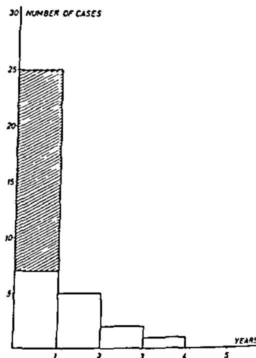


Fig. 5. Time of occurrence of lymph node metastases in 33 patients. ▨ primary □ secondary metastases

diation of a glottic T3 N0 tumour, field size 6 cm × 6 cm. A recurrence 9 months later led to total laryngectomy but a neoplastic infiltration of the soft tissues, size 3 cm × 4 cm, developed, adhering to the left side of the pharynx. Electron radiation by a 20 MeV linear accelerator was administered: 500 rad twice weekly, totally 6 000 rad in 39 days, field size 8 cm × 10 cm. The calculated minimum tumour dose was 5 700 rad. The irradiation was well tolerated but caused severe atrophy of the skin and underlying tissues at the site of the primarily treated field but no necroses. The patient is free of recurrence more than 7 years after the onset of the disease and 4.5 years after the electron radiation.

Lymph node metastases. On first admission 11 per cent (17/152) presented lymph nodes assumed to be involved (Table 1). One case may be added in which routine exploration of the regional lymph nodes at the primary total laryngectomy revealed metastases, thus 12 per cent (18/152) had primary

Table 8

Results of treatment of regional lymph node metastases

	Primary	Secondary	All cases
5 year survival			
Number of cases	18	15	33
Irradiation, curative	6/8*	1/1**	7/9
Irradiation palliative	0/1	0/3	0/4
Irradiation + neck dissection	1/6		1/6
Neck dissection	2/3	3/8**	5/11
No treatment		0/3	0/3
Total	9/18	4/15	13/33
Crude 5 year survival rate***	50%	27%	39%

* 1 patient alive with recurrence of local tumour
** 1 patient alive with inoperable regional lymph node metastases
*** Calculated from the first day of treatment of the primary local tumour

metastases in the regional lymph nodes Late nodal involvement *occurred in* 15 cases, all within the first 4 years after treatment (Fig 5) Thus, 33 patients, or 22 per cent, had verified or presumed lymph node metastases The frequency was highest *among the supraglottic* tumours, with 11 primary and 8 late metastases, i e 46 per cent (19/41) The metastases were located in the jugulo-digastric nodes in 19 cases, in 4 in the jugulo-omohyoid nodes, and in 7 cases in lymph nodes between these two groups Only in one case were both groups involved One patient had a prelaryngeal metastasis and also metastases in the contralateral supraclavicular region, and one only *in the mediastinum* This was the mentioned T1 NO MO glottic case but co-existing malignant disease could not be excluded with certainty Twenty-five patients had involvement of homolateral lymph nodes, 6 bilateral and 2 contralateral involvement (one of these latter patients was the one with a prelaryngeal metastasis)

Primary full dose irradiation was used in 14 cases (Table 8) *Of these* 6 survived for more than 5 years, 8 developed recurrence in the irradiated area, 2 were inoperable Six underwent operation including neck dissection, one survived for more than 5 years, one died of *distant metastases* without recurrence in the neck and 4 of recurrence in the neck Table 8 also reveals that in 3 patients primary neck dissection was carried out, which proved curative in 2

Late metastases *occurred in* 15 cases, in 3 of these within the field irradiated at the treatment of the primary tumour and in 12 outside this field Eight under-

went neck dissection, and 3 of them survived for more than 5 years. One patient was given 15 MeV electron irradiation, 6 000 rad/35 days, without success. In 6 cases curative treatment was out of the question. The 5-year crude survival was 39 per cent (13/33) (Table 8).

The entire material includes 19 neck dissections, 12 in connection with total laryngectomy and 7 separately. Microscopy of the lymph node specimens revealed only inflammatory changes in 2 cases (not included in the group of metastases).

Neck dissection in connection with total laryngectomy was performed through a supplementary incision towards the acromion. The 7 separate dissections were performed through a superior horizontal arcuate incision extending from the mastoid process to the submental region. From the middle a vertical incision was made, split distally into Y shape, into an anterior and a posterior part. After the skin had been deflected, all tissue between the platysma and the muscle fascia was removed, including the sternocleidomastoid muscle, the omohyoid muscle, and the internal jugular vein, but preserving the carotid artery and vagus nerve.

Recurrences following neck dissection were tentatively treated in 2 cases by full dose irradiation. One of these patients developed a 4 cm × 4 cm × 3 cm conglomerate of lymph nodes in the supraclavicular region. ^{60}Co irradiation was administered with a minimum tumour dose of 4 800 rad in 23 days. The patient died, free of recurrence, 8 years after this treatment and 9 years after the onset of the disease. The other patient developed a large soft-tissue recurrence after primary laryngectomy with neck dissection. Curative ^{60}Co irradiation was tried but without success. The remaining patients with recurrences received various forms of palliative treatment.

In the 19 metastatic supraglottic cases no cure of the nodal recurrences appearing after primary irradiation was obtained.

Operative complications. After 39 total laryngectomies hypopharyngeal fistulas occurred in 8 cases, all primarily irradiated. Six healed in a few weeks during which the patients were nourished through a gastric tube. Two cases were protracted and were operated upon. Major, but not serious infections occurred in 3 cases. There was no instance of major necroses.

Vocal function after radiation therapy and surgery. From Table 4 it is apparent that 74 patients survived for more than 5 years after irradiation alone, with a normal or almost normal voice in 84 per cent (62/74), less satisfactory but adequate voices in 16 per cent (12/74). Two of these later developed recurrences. The totally laryngectomized cases have previously been discussed by JENSEN & BALSLEV (1967), who reported that 60 per cent obtained a satisfactory oesophageal voice.

Prognosis The prognosis was clearly better for women than for men, the 5-year crude survival rate among the females being 88 per cent (14/16) as compared with 68 per cent within the whole series (104/152) The observed difference is 2 1 times the standard error, i e significant It should be pointed out that the staging revealed no difference between the two sexes

The prognosis within the various age groups was estimated, a correction being made for deaths from causes other than carcinoma of the larynx This revealed that the prognosis was relatively poorer in the older age groups than in the younger ones but the difference was not significant

No significant relationship was found between recurrence and late lymph node metastases The frequency of late metastases among the supraglottic cases was exactly the same with and without local recurrence

As stated, the material was re-classified, which disclosed 3 additional supra glottic cases situated at the marginal zone of the aditus laryngis (AJC, UICC 1972) They were classified as one T1a NO MO and two T4 NO MO All three were primarily irradiated One is free of recurrence after the irradiation, one after irradiation followed by total laryngectomy, and one developed an in curable pharyngeal recurrence These three cases do not alter the survival calculations however

Discussion

Up to 1972 two closely related systems, UICC (1968) and AJC (1962), existed for the classification of laryngeal carcinoma Contrary to UICC, AJC included the marginal zone of the aditus laryngis, and had rules for staging The UICC and AJC rules published in 1972 are identical and mark a great advance The T classification is now more logical and pays more regard to the way in which the laryngeal carcinoma spreads Previously, a case would be transferred from the T1 to the T3 group if a glottic tumour extended only slightly into the subglottis or into the bottom of the ventricle Such a case will now be classified as T2 Previously a glottic case with fixation of the vocal cord was classified as T2 However, it has consistently been demonstrated that fixation of the vocal cord means a poorer prognosis, and from this point of view such cases belong to T3 (HEISE & BAYLIS 1966, JORGENSEN 1970) Rules were also laid down for staging and for grouping by presumed site of origin Both are of essential importance in characterizing a material Comparison between different series is thus improved

The present series, classified into a supraglottic, glottic and subglottic group and in accordance with the TNM system, does not seem to differ from most other series published in recent years (LEDERMAN 1970, PUTNEY & CHAPMAN

1972, SMITH et coll 1973) except for three which differ from all others in a preponderance of supraglottic cases Thus, LAUFRMA (1967) and TASKINEN (1969), in large Finnish series, found two-thirds of the cases to be supraglottic, and IWAMOTO (1971) in a Japanese series of 2 160 cases 55 per cent In connection with the revision of the TNM system SMITH et coll (1973) published an American series of 1 645 cases They found good agreement between the groups of the revised TNM system and their prognosis As is evident from Tables 5 and 6 the same applies to the present series

The present material has previously been published with a cumulative calculation of the prognosis (JØRGENSEN & SELL 1971) using the UICC rules (1968) valid at that time, but has now been re classified (Table 1) In the revision 3 additional cases were found, considered as having arisen from the marginal zone One was an extensive T4 case difficult to classify exactly Possibly it had arisen in the vallecula epiglottica but it was decided to include it in the present series The other two were small, one affecting only the marginal zone (T1 case), the other one the lateral aspect of the epiglottic margin with slight infiltration into the pharyngo-epiglottic fold This case was classified as T4, the rules being strictly followed Obviously, it was grouped in an advanced stage, and although such cases are relatively rare, there ought to be rules for classifying cases localized at the junction of anatomic regions This problem is of course of topical interest concerning all transitional zones The division of N1 and N2 cases into a and b subgroups according to whether or not any regional lymph nodes are considered to be involved proved worthless in the present series Such a grouping can acquire value only when the TNM system is used for its true object, viz for prospective analyses

As to the aetiology, no information is obtainable from the present series, as data concerning smoking habits were available for only 46 per cent of the patients WYNDER et coll (1956) and STELL (1972) demonstrated a distinct relationship between smoking and the occurrence of laryngeal carcinoma

The calculation of survival for the entire series gave a crude 5 year survival of 68 per cent By a cumulative survival calculation (Fig 2) it was possible, as early as the end of 1969—or 4 years before all the patients had been observed for 5 years—to submit an expected 5-year result between 65 and 70 per cent

The method used (NOHRMAN 1953, JØRGENSEN & SELL 1971) has thus proved highly applicable also in the present material It is based upon the same principles as the actuarial method given by UICC in 1969

The therapeutic result should be assessed for the entire material as well as on the basis of detailed analyses of the subgroups The total result may be assessed on the following factors 5-year survival, frequency of total laryngectomies, complications after radiation therapy and supplementary surgery, and vocal

function A 68 per cent survival is comparable to other reports The frequency of total laryngectomies is low, 20 per cent (21/104) This means that 80 per cent of the patients with more than 5 years' survival had preserved laryngeal function This is due partly to the majority of the patients having been cured after irradiation only and the frequency of conservative surgery on the larynx being quite high, viz 7 per cent (10/152) Complications of irradiation were few, and left only a few sequelae, only one patient had to be tracheotomized Normal or almost normal ability of speech was retained by 84 per cent (62/74) of the patients who received radiation therapy only and who survived longer than 5 years The remaining 16 per cent (12/74) had minor speech disturbances—all assessed at the 5 year limit The surgical principles after irradiation were the same as at primary surgery No fatal complications occurred and only in 8 cases pharyngo-cutaneous fistulas

The treatment, primary irradiation and operation on residual tumours or recurrences gave a satisfactory overall result Assessment of the subgroups, however, discloses a different picture Whereas the 5-year result was satisfactory in the glottic group (Table 5), the survival in the supraglottic group was relatively low, 44 per cent (Table 6) The frequency of regional lymph node metastases was high in the supraglottic group, 46 per cent (19/41), and this influences the prognosis unfavourably Thus, a more active attitude to the metastases might be expected to improve the results In evaluating the treatment of metastases (Table 8) the impression might be gained that primary irradiation had a favourable effect However, in at most 70 to 80 per cent of the lymph nodes clinically assumed to contain metastases microscopy did reveal malignant tissue (McGavran et coll 1961), and 3 or 4 cases must therefore be deducted from the 15 irradiated patients of the present material These cases must me expected to appear mainly in the group of cured patients This presumably leaves only 2 or 3 patients with regional lymph node metastases cured by irradiation, as 8 developed recurrences, and of these only one was cured by neck dissection The presumption that irradiation has little prospects of curing manifest metastases from laryngeal carcinoma is supported by experience from other malignancies of the head neck region (Jorgensen et coll 1973, Elbrønd et coll 1973) as well as by the experience from the treatment of laryngeal carcinoma during the preceding period (Jorgensen 1970) The numbers are small, but suggest another treatment of cases with lymph node metastases present primarily Such cases should be treated by full dose irradiation to the local tumour and lymph node metastases, included in the same field, as well as 6 to 8 weeks after the end of irradiation, neck dissection—bilateral only if the contralateral lymph nodes possibly are metastatic primarily The results not only in the supraglottic group but also in more advanced glottic or subglottic cases may thus be improved

Recent reports support the assumption that such a combined treatment is rational In a material of 64 cases of extensive laryngeal carcinoma, 44 with palpable lymph nodes primarily, irradiation (5 500 rad/5 weeks) was administered, and 3 weeks later total laryngectomy + neck dissection performed (GOLDMAN et coll 1972) The surgery was bilateral if necessary In 32 cases the cervical nodes contained carcinomatous tissue The recurrence rate in the cervical nodes was low, 9 4 per cent FLETCHER et coll (1970) reported a large series of supraglottic cases treated by primary neck dissection Thirty-eight had full dose postoperative irradiation, whereas 55 received no postoperative treatment The recurrence rate in the cervical nodes was 10 5 per cent and 36 per cent, respectively In the contralateral nodes only one recurrence occurred in the irradiated group, but 13 in the other

MCGAVRAN et coll (1961), in a series of primarily operated supraglottic or transglottic cases, found that 48 per cent either had or developed contralateral lymph node metastases while BILLER et coll (1971) found the frequency of *bilateral or contralateral node metastases in primary neck dissection* of supraglottic cases to be 24 per cent (33/140) Among the patients who underwent ipsilateral neck dissection late contralateral recurrences occurred in 41 per cent (20/49) These reports definitely support the opinion that irradiation has an effect in inhibiting the occurrence of contralateral lymph node metastases

BRYCE (1972) also emphasized the unsatisfactory results of irradiation of supraglottic tumours but stressed mainly the tendency to local recurrence He recommended supraglottic laryngectomy primarily in all cases if technically possible and obtained thereby improved therapeutic results, primary full dose irradiation was recommended in other cases BRYCE suggests that all T4 cases be treated by combination of irradiation and surgery because of the slight prospects that only irradiation can cure these cases Surgery may be used to deal with lymph node metastases, if present Primary surgery in selected supraglottic cases was also recommended by OLOFSSON et coll (1972) An essentially different treatment plan was suggested by FLETCHER et coll (1970) primary irradiation for small supraglottic tumours, laryngectomy for large infiltrating tumours followed by postoperative irradiation

DIFFENBACH & PHILLIPS (1972) also advocated primary radiation therapy in supraglottic cases, and they reported good results Most North American authors, however, recommend primary surgery in the treatment of supraglottic cases, emphasizing the importance of conservative procedures (SHUMRICH 1969, 1971, SOM 1970, LEONARD & LITTON 1971, OGURA 1972)

The principle for treating carcinoma of the larynx still differ widely, there being every transition from primary surgery to primary irradiation PUTNEY & CHAPMAN (1972), in a series of 311 unselected cases treated primarily by surgery,

reported an extremely satisfactory survival result However, total laryngectomy had been carried out in 58 per cent Selected series of primarily irradiated patients (JESSE et coll 1971, PEREZ et coll 1971), have shown that in several clinics the attitude at present is towards primary irradiation of glottic T1 and T2 cases, surgery being reserved for the more advanced stages SISSON et coll (1970), BILLER et coll (1971), LEONARD et coll (1972) and OGURA (1972) emphasized the good survival results following primary surgery, and accordingly they recommended primary radiation therapy only for glottic T1a cases Applying similar principles KORNMESSER & TROCEANU (1970) obtained favourable therapeutic results

IWAMOTO (1971) reported a series of 2 160 patients, in the majority of cases the primary treatment was surgery LEDERMAN (1970, 1971) used in all cases primary irradiation but combined with surgery if the cartilage was invaded or penetrated, or if lymph node metastases were present If the vocal cord was fixed, and if the response was not satisfactory after 4 000 rad/4 weeks, the irradiation was interrupted and the patient operated upon 3 weeks later Primary radiation therapy was considered the method of choice by KERR et coll (1970) but possibly as a link in combined treatment of more advanced cases Large series in which the predominant treatment was primary irradiation were reported by MÅRTENSON (1967) and TASKINEN (1969) Postoperative radiation therapy significantly reduced the number of recurrences of local tumours as well as in the cervical lymph nodes (FLETCHER et coll 1970, TASKINEN 1969)

Conclusion

The present series has shown that primary irradiation and surgery of a residual tumour, if any, or of a recurrence afforded good results in the glottic group in which the 5 year survival was 80 per cent, the frequency of total laryngectomies and complications was low In the supraglottic group the survival was only 44 per cent, possibly due to the high frequency of metastases in the regional lymph nodes (46 per cent) On the basis of reports in the literature and of the present results, a combination of irradiation and surgery in the treatment of patients with primary lymph node metastases is suggested This schedule is presumed to improve the results mainly in the supraglottic group Generally, an increasing trend towards primary irradiation may be observed but it is also evident that divergent opinions on the treatment of laryngeal carcinoma still exist

SUMMARY

A material of 152 cases of laryngeal carcinoma observed for more than 5 years is reported Among these 147 patients were primarily treated by ^{60}Co irradiation and 5 by surgery The 5 year crude survival in the total series was 68 per cent In the supraglottic group the

frequency of regional lymph node metastases was 46 per cent and the 5-year crude survival 44 per cent. A combination of irradiation and surgery for patients with lymph node metastases present primarily is suggested

ZUSAMMENFASSUNG

Es wird uber ein Material von 152 Fallen mit einem Larynx Karzinom, das langer als 5 Jahre beobachtet worden war, berichtet; 147 Patienten waren primar mit ^{60}Co Bestrahlung und 5 chirurgisch behandelt worden Die 5-Jahres Überlebensrate der gesamten Gruppe betrug 68 % In der subglottis Gruppe betrug die Frequenz regionaler Lymphknoten Metastasen 46 % und die Überlebensrate 44 % Eine mit Bestrahlung kombinierte Therapie fur Patienten mit Lymphknoten Metastasen wird vorgeschlagen

RÉSUMÉ

Présentation d'une série de 152 cas de cancer du larynx observés pendant plus de 5 ans Cent quarante sept malades ont été traités primitivement par irradiation au ^{60}Co et 5 par chirurgie Le taux de survie brute à 5 ans a été dans l'ensemble de la serie de 68 % Dans le groupe des cancers sus-glottiques, la fréquence des métastases aux ganglions lymphatiques régionaux a été de 46 % et le taux brut de survie à 5 ans a été de 44 % Les auteurs proposent l'association d'irradiation et de chirurgie pour les malades qui ont des métastases ganglionnaires lymphatiques lors du premier examen

REFERENCES

AJC American Joint Committee on Cancer Staging and End Results Reporting Clinical staging system for carcinoma of the larynx Chicago 1962, 1972

Biller H F, Ogura J H and Lindsay L Hemilaryngectomy for 12 glottic cancers Arch Otolaryng 93 (1971), 238

— Davis W H and Ogura J H Delayed contralateral cervical metastases with laryngeal and laryngopharyngeal cancers Laryngoscope (St Louis) 81 (1971), 1499

Bryce D P The role of surgery in the management of carcinoma of the larynx J Laryng 86 (1972), 669

Deffebach R R and Phillips T L Role of radiation therapy in the treatment of supraglottic carcinoma Cancer (Philad) 30 (1972) 1159

Elbrond O, Andersen A P and Jorgensen K Carcinoma linguae A series of 96 cases Acta radiol Ther Phys Biol 12 (1973), 465

Fletcher G H, Jesse R H, Lindberg R D and Koons C R The place of radiotherapy in the management of the squamous cell carcinoma of the supraglottic larynx Amer J Roentgenol 108 (1970), 19

Goldman J L Zak F G, Roffman J D and Birken E A High dosage preoperative radiation and surgery for carcinoma of the larynx and laryngopharynx Ann Otol (St Louis) 81 (1972), 488

Heise H and Baylis P Clinical staging in cancer of the larynx a report on 788 cases using the American Joint Committee's method of stage classification J nat Cancer Inst 36 (1966), 45

Iwamoto H Cancer of the larynx in Japan Laryngoscope (St Louis) 81 (1971), 387

JENSEN T S and BALSLEV A 104 tilfaelde af laryngectomia totalis Med specielt henblik på følgetilstanden (In Danish) Nord Med 78 (1967), 1057
JESSE R H LINDBERG R D and HORIOT J C Vocal cord cancer with anterior commissure extension Amer J Surg 122 (1971) 437
JORGENSEN K Carcinoma of the larynx I Treatment mainly by primary irradiation Acta radiol Ther Phys Biol 9 (1970) 401
— and SELL A Carcinoma of the larynx II Treatment by ^{60}Co supervoltage irradiation Acta radiol Ther Phys Biol 10 (1971), 161
— ELBROND O and ANDERSEN A P Carcinoma of the lip Series of 869 patients Acta radiol Ther Phys Biol 12 (1973) 177
KERR R C MADIGAN J P and MILLAR H S Carcinoma of the larynx in Melbourne, 1956–1965 Aust N Z J Surg 40 (1970) 19
KORNMESSER H J und TROCEANU M Unsere Behandlungsergebnisse beim Kehlkopfkarzinom Z Laryng Rhinol 49 (1970), 444
LAUERMA S Treatment of laryngeal cancer A study of 638 cases Acta oto-laryng (1967) Suppl No 225
LEDERMAN M Radiotherapy of cancer of the larynx J Laryng 84 (1970), 867
— Cancer of the larynx Part I Natural history in relation to treatment Brit J Radiol 44 (1971) 568
LEONARD J R and LITTON W B Selection of the patient for conservation surgery of the larynx Ann Otol (St Louis) 80 (1971), 232
HOLT P and MARAN A G Treatment of vocal cord carcinoma by vertical hemilaryngectomy Ann Otol (St Louis) 81 (1972) 469
MCGAVRAN M M, BAUER C and OGURA J H The incidence of cervical lymph node metastases from epidermoid carcinoma of the larynx and their relationship to certain characteristics of the primary tumor Cancer (Philad.) 14 (1961), 55
MÅRTENSSON B Transconioscopy A method for improvement of endolaryngeal diagnosis Pract oto rhino-laryng (Basel) 29 (1967) 217
NORRMAN B A Survival rate calculation Acta radiol Ther Phys Biol 39 (1953) 78
OGURA J H Selection of patients for conservation surgery of the larynx and pharynx Transactions of the American academy of ophthalmology and otology 76 (1972), 741
OLOFSSON J WILLIAMS G T BRYCE D P and RIDER W D Radiotherapy vs conservation surgery in the treatment of selected supraglottic carcinomas Arch Otolaryng 95 (1972), 240
PEREZ C A MILL W B OGURA J H and POWERS W E Irradiation of early carcinoma of the larynx Arch Otolaryng 93 (1971), 469
PUTNEY F J and BAGLEY C S The two stage technique of laryngectomy Ann Otol (St Louis) 79 (1970) 1057
and CHAPMAN C E Carcinoma of the larynx Analysis of 311 cases treated surgically Ann Otol (St Louis) 81 (1972), 455
SHUMRICK D A Carcinoma of the supraglottis and tongue treated by supraglottic laryngectomy and mandibular swing Laryngoscope (St Louis) 79 (1969), 1443
Partial laryngectomy Amer J Surg 122 (1971) 440
SISSON G A GOLDSTEIN J C and BECKER G D Surgery of limited lesions of the larynx (past and present) Otolaryng Clin N Amer 3 (1970), 579
SMITH R R CAULK R FRAZELL E HELINGER P H, MACCOMB W S, RUSSELL W O, SCHULZ M D and TUCKER G F Revision of the clinical staging system for cancer of the larynx Cancer (Philad) 31 (1973) 72

SOM M L Conservation surgery for carcinoma of the supraglottis J Laryng 84 (1970), 655
STELL P M Smoking and laryngeal cancer Lancet I (1972), 617
TASKINEN P J Radiotherapy and TNM classification of cancer of the larynx Acta radiol (1969) Suppl No 287
UICC *Union Internationale Contre le Cancer, Geneva 1968, 1972*
UICC TNM General rules, Geneva 1969
WYNDER E L, IRWIN J and DAY E A study of environmental factors in cancer of the larynx Cancer (Philad) 110 (1956), 86

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EFFECT OF GAMMA IRRADIATION ON THE BONE MARROW

Determination by means of ^{198}Au

NILS ERIK SATERBORG

After intravenous injection of a colloidal substance a fraction of the colloid is incorporated by the reticuloendothelial cells (RE cells) of the bone marrow. This uptake can be analysed in vivo by the use of radioactive colloids (^{198}Au or ^{99}Tc^{m} sulfur colloid). Various bone marrow diseases may modify the uptake (LARSSON & JONSSON 1957, ENGSTEDT et coll 1958, LARSSON et coll 1963, EDWARDS et coll 1964, KNISELEY et coll 1964), after irradiation of the marrow a decrease was observed (LARSSON et coll 1963, KJELLGREN & JONSSON 1969).

Histologic changes in the bone marrow have been reported both after general irradiation (FLIEDNER et coll 1955, 1961), as well as after local irradiation (GOSWITZ et coll 1963, KOLLATH 1963, KNOSPE et coll 1966, 1968). A comparison between the histologic appearance and the modification of the uptake of colloidal ^{198}Au in the bone marrow after local irradiation appeared to be of interest.

The phagocytic and proliferative abilities of the RES have been reported to respond differently to irradiation. Recent investigations in rats seem to indicate

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that phagocytosis is enhanced by irradiation, as measured by the intravascular clearance of carbon, even when sublethal doses are used (ANTONIJEVIC 1968, FLEMMING et coll 1970, FRED et coll 1970) The proliferative capacity of the RES in whole body irradiated mice was reported to be depressed during the first days after irradiation (WOOLES et coll 1962, FRED et coll 1970) but after about seven days a rapid restoration occurred (FRED et coll) A latent injury, not detectable by the colloidal carbon clearance method was temporarily present It was revealed by injections of glucan which gave a general proliferation of the RES in the unirradiated but not in the irradiated animals

Stimulation of the RES of the bone marrow may also be obtained by phenylhydrazine-hydrochloride which induces hemolysis, increased hematopoiesis as well as increased uptake of gold colloid (SATERBORG 1974) In the present investigation phenylhydrazine treatment was used in order to determine the proliferative capacity of the RES of the bone marrow after irradiation

Material and Methods

During ether anesthesia the right femur of 398 rats (mean weight about 200 g) was locally irradiated with a ^{60}Co apparatus containing about 4 000 Ci and with a SSD of 60 cm Single doses of 1 000 rad, 2 500 rad and 4 000 rad were given (Table) Each rat was protected by a 40 mm thick lead shield with a 15 mm × 35 mm slit through which the right femur was irradiated The length of the slit corresponded approximately to the length of a rat femur, femurs longer than the slit were not used Before and after the irradiation the localization of the femur in the slit was controlled with an image intensifier The dose was controlled by ionizing chambers placed centrally in a plexiglas phantom conforming to the dimensions of a rat femur

As controls 68 unirradiated rats were anesthetized in the same way as the irradiated and later on killed at varying times after the anesthesia In addition 31 rats neither irradiated nor anesthetized were also examined

The irradiated animals were killed after 1, 2, 4, 10, 20, 30, 60, 90 and 120 days, four animals given 2 500 rad after 10 months (Table)

Two hours before the animals were killed with ether 100 μCi ^{198}Au colloid (specific activity 0 5 to 1 mCi/ml) were injected in the tail vein The right and left femur were excised and liquefied in 5 ml conc HCl The amount of ^{198}Au in these samples was measured in a well crystal scintillation counter, compared with a reference and expressed as percentage of the injected activity

Bone marrow specimens both from the irradiated and the unirradiated femur were taken from the various groups by curettage The femur was split, marrow taken from the diaphysis and fixed in alcohol formalin, paraffin embedded,

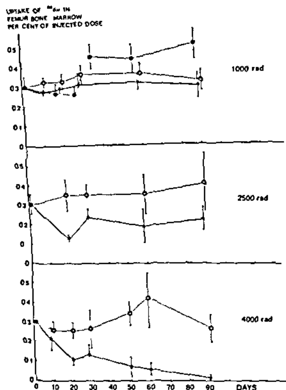


Fig 1 Uptake of colloidal ^{198}Au in the femur bone marrow of rats after local irradiation Uptake expressed in per cent of injected dose ■ normal animals and controls (anesthetized but unirradiated), ○ left (unirradiated) femur, ★ right (irradiated) femur

sectioned and stained with hematoxylin-eosin The histology of the bone marrow was recorded in semi quantitative terms by ordinary light microscopy and the amount of hematopoietic cells, the number of fat cells and the degree of the stromal reaction was estimated

Fourteen rabbits weighing 2 500 to 3 000 g were irradiated with a single dose of 1 000 rad to the whole caudal part of the body with exception of the left tibia and the distal part of the left femur, which were shielded Two days later the whole cranial part of the body was given a single dose of 1 000 rad The pause between these two irradiations was necessary in order to avoid radiation induced mortality during the experimental period The dose was controlled by ionization chambers placed in front of the irradiated extremity

Seven of the irradiated rabbits received at the first irradiation 1 ml of phenylhydrazine 2 5 % subcutaneously and then each day until they were killed after 7, 9, 13, 14, 17 and 21 days Seven irradiated but not phenylhydrazine treated animals were killed after 14, 15, 17, 20, 23, 25 and 26 days In all these animals

Table

Uptake of colloidal ^{198}Au in rat femur bone marrow Number of recordings and relation between uptake on irradiated and unirradiated sides varying times after irradiation of right femur

Time after irradiation	Dose		
	1000 rad	2500 rad	4000 rad
1 days	1 18±0 07 n = 14	1 13±0 09 n = 28	0 98±0 14 n = 10
2 "	0 98±0 08 n = 14	1 02±0 20 n = 21	0 83±0 10 n = 9
4 "	1 08±0 07 n = 10	0 91±0 12 n = 23	0 78±0 09 n = 9
10 "	0 81±0 04 n = 10		0 81±0 15 n = 10
20 "	0 87±0 06 n = 5	0 39±0 11 n = 6	0 43±0 11 n = 6
30 "	0 88±0 08 n = 11	0 67±0 07 n = 10	0 52±0 19 n = 13
50 "			0 24±0 16 n = 13
60 "	0 92±0 09 n = 11	0 54±0 15 n = 25	0 17±0 09 n = 17
3 months	0 92±0 14 n = 17	0 45±0 09 n = 27	0 11±0 08 n = 21
4 "	0 97±0 07 n = 7	0 52±0 09 n = 12	0 18±0 13 n = 24
6 "	0 85±0 08 n = 4		0 33±0 09 n – 7
10 "		0 83±0 05 n = 4	

Controls 0 99±0 06 n = 31

and in six untreated rabbits the uptake of ^{198}Au colloid in the tibia bone marrow was measured using the same technique as for the rats The rabbits were killed by nembutal plus air insufflation through the ear vein

All mean values are given with one standard deviation For comparison of the mean values the Wilcoxon test for unpaired observations was used

Results

The results of the irradiation of rat femur are presented in Figs 1 to 4 and in the Table After 1 000 and 2 500 rad but not after 4 000 rad a transitory increase of the uptake was observed after one day (Fig 2) The increase was statistically significant ($p < 0 01$) The histologic appearances of the bone marrow agreed with those reported by KNOSPE et coll (1966) after local irradiation with supralethal doses Thus, dilatation of the bone marrow vessels combined with areas of bleeding into the parenchyma occurred in the irradiated femur during the first few days and the number of cells decreased up to 4 to 5 days after the irradiation

After 2 500 and 4 000 rad the uptake of colloidal ^{198}Au continually decreased up to 20 days followed by a slight increase between 20 and 30 days after the

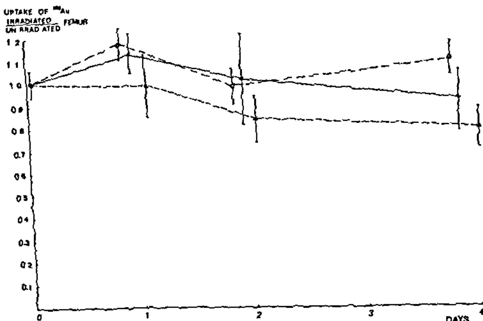


Fig 2 Uptake of colloidal ^{198}Au in the femur bone marrow of rats 0 to 4 days after local irradiation Uptake expressed as quotient between uptake in irradiated and unirradiated femur ○ 1 000 rad ★ 2 500 rad ▲ 4 000 rad

irradiation (Figs 1, 3) The increase was statistically significant with 2 500 rad ($p < 0.01$) but not with 4 000 rad Histologically only small variations in the hematopoietic cell or flat cell content of the bone marrow were indicated The hematopoietic cell content was about the same as in the normal bone marrow 30 days after 2 500 or 4 000 rad

The uptake of colloidal ^{198}Au rapidly decreased 30 days after both 2 500 and 4 000 rad (Fig 3) The number of hematopoietic cells was markedly reduced Three months after 4 000 rad no hematopoiesis could be established and beginning fibrosis was observed Three months after 2 500 rad the number of cells was greater than after 4 000 rad and some hematopoiesis could still be found Furthermore, after 2 500 rad there was later an increase in the uptake of colloidal ^{198}Au (Fig 4), at 10 months the uptake was only slightly depressed compared with the normal values ($p < 0.01$) At this time fibrosis had developed in a large part of the femur but also areas of hematopoietic regeneration in the subendosteal parts occurred

Table

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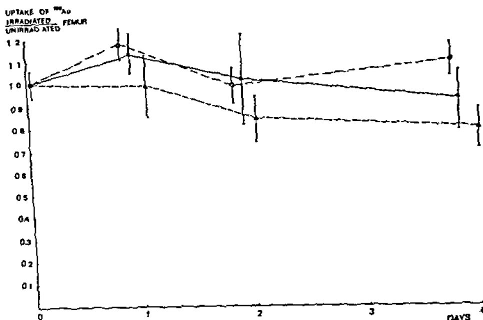


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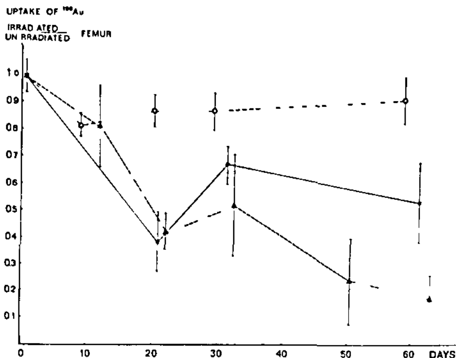


Fig. 3. Uptake of colloidal ^{198}Au in the femur bone marrow of rats 0 to 60 days after local irradiation. Uptake expressed as quotient between uptake in irradiated and unirradiated femur. ○ 1 000 rad ★ 2 500 rad ▲ 4 000 rad.

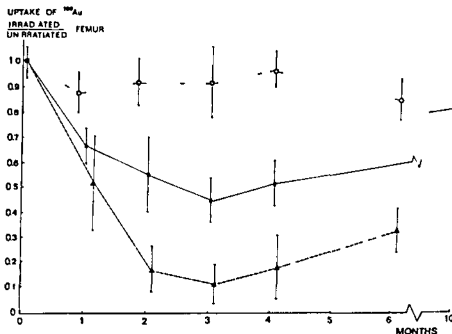


Fig. 4. Uptake of colloidal ^{198}Au in the femur bone marrow of rats 0 to 6 months after local irradiation. Uptake expressed as quotient between uptake in irradiated and unirradiated femur. ○ 1 000 rad ★ 2 500 rad ▲ 4 000 rad.

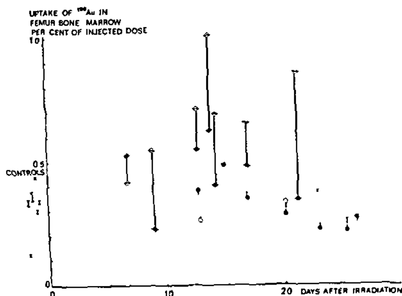


Fig 5 Uptake of colloidal ^{198}Au in the tibia of 14 rabbits at various times after whole body irradiation with 1 000 rad with exception of left tibia Seven of the rabbits were after irradiation treated with 1 ml phenylhydrazine 2 5 % daily As a comparison the uptake of colloidal gold [illegible] |——| = uptake in the [illegible] the tibia of seven irradiated [illegible] n the tibia of seven irradiat [illegible] ● Uptake in irradiated tibia
○ = uptake in unirradiated tibia

After 1 000 rad the initial increase in the uptake of colloidal ^{198}Au was followed by a slight but statistically significant decrease (Fig 3) recorded after 10 and 20 days ($p < 0\,01$) as well as after 30 days ($p < 0\,05$) With the exception of a moderate depletion of hematopoietic cells in the bone marrow during the first few days after irradiation no histologic changes were observed in the series irradiatd with 1 000 rad

The results of the rabbit series are illustrated in Fig 5 In the seven rabbits given 1 000 rad 'whole body irradiation' (left tibia shielded) only statistically insignificant differences were recorded between the uptake of colloidal ^{198}Au in right and left tibia during an observation period of 26 days In the seven rabbits similarly irradiated and phenylhydrazine treated for varying days after irradiation, the uptake of colloidal ^{198}Au in the unirradiated femur was considerably increased ($p < 0\,01$) The uptake in the tibia of the irradiated and phenylhydrazine treated animals was not statistically significantly increased compared

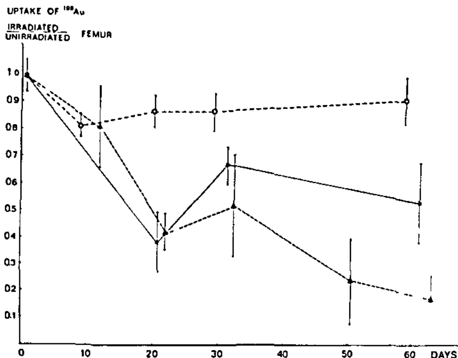


Fig 3 Uptake of colloidal ^{198}Au in the femur bone marrow of rats 0 to 60 days after local irradiation Uptake expressed as quotient between uptake in irradiated and unirradiated femur ○ 1 000 rad, ★ 2 500 rad, ▲ 4 000 rad

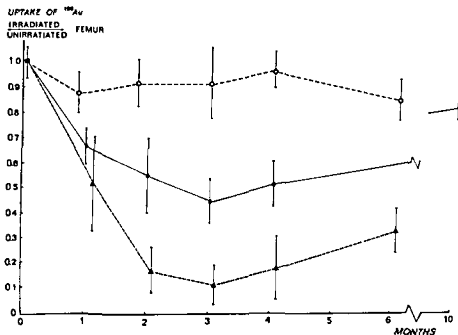


Fig 4 Uptake of colloidal ^{198}Au in the femur bone marrow of rats 0 to 6 months after local irradiation Uptake expressed as quotient between uptake in irradiated and unirradiated femur ○ 1 000 rad, ★ 2 500 rad, ▲ 4 000 rad

no significant increase after 4 000 rad The increased uptake after irradiation with 1 000 and 2 500 rad may be due to increased phagocytic activity of the individual cells but might also be explained by the hyperemia of the bone marrow which is a regular early reaction after irradiation (BOND et coll 1962) KOLLATH (1963) and KNOSPE et coll (1966, 1968), described a decrease in the number of bone marrow cells occurring in two stages after sublethal doses Initially a depopulation of parenchymal cells during the first 3 to 4 days occurred, followed by regeneration after about one week The resulting number of bone marrow cells was dependent upon dosage After sublethal or lethal doses, a secondary decrease of the cell content occurred beginning after about one month This decrease in cell number is most likely the result of the destruction of the bone marrow stroma, which contains slowly proliferating RE cells (HARRIS et coll 1963, CAFFREY et coll 1966) ending in bone marrow fibrosis The increased uptake of ^{198}Au colloid after 30 days compared with 20 days which occurred with 2 500 rad in the present investigation may be due to regenerative phenomena occurring before the final cell death of the slowly multiplying stromal bone marrow cells That a compartment of slowly multiplying cells is of importance for regenerative processes in the bone marrow has been demonstrated by HAAS et coll (1972)

Local mechanical injury to the bone marrow (curettage) induces blood vessel regeneration in the uninjured parts and the newly formed vessels penetrate the injured areas (BRÅNEMARK et coll 1964, AMSEL et coll 1969) It is suggested that similar events also occur in the bone marrow injured by radiation It seems also probable that this type of vascular regeneration is of fundamental importance for a succeeding repopulation of the marrow by migrating hematopoietic cells (TAVASSOLI & WEISS 1971)

The uptake of colloidal ^{198}Au decreased rapidly after 4 000 rad which is consistent with the marked histologic changes After 2 500 rad a relatively high uptake of colloid was recorded ten months after irradiation indicating regeneration of the *sinusoids* The histology revealed a centrally rather acellular marrow with subendosteal areas of hematopoietic regeneration

NELP et coll (1970) found a considerable depression of the uptake of ^{99}Tc^{m} sulfur colloid *in the tibia* and fibula of rabbits 60 days after irradiation with 1 000 R (250 kV) Their results may not be directly comparable to the present results as the delivered ^{60}Co doses were given in units of rad Differences in fluences and ranges of the electrons set in motion by roentgen and ^{60}Co gamma rays may better explain the more destructive effect upon the bone marrow after 1 000 R compared with the small effect after 1 000 rad ^{60}Co gamma rays (ICRU report 10d, 1963)

Neither stimulation nor depression of the uptake of ^{198}Au colloid was observed

with the non-phenylhydrazine treated. *The difference in uptake between the irradiated and the unirradiated rabbit tibia was considerably greater in the phenylhydrazine treated series than in the control animals* ($p < 0.01$).

Discussion

Colloidal gold accumulates in the sinusoidal lining cells of the bone marrow which reflects the physiologic state of these cells and the number of patent sinusoids of the marrow (SATERBORG 1974). The uptake of injected colloidal ^{198}Au in the pelvic bone marrow was permanently depressed after fractionated irradiation of the pelvis with 4 000 rad given in about 25 fractions (6 per week) covering a period of 50 days (KJELLGREN & JONSSON 1969). Depressed uptake of ^{99}Tc^{m} sulfur-colloid could not be demonstrated by NELP et coll. (1970) during the first few days after local irradiation of the bone marrow. They demonstrated that the structures of the sinusoids were intact during the same time after the intravenous injection of carbon particles. The reticuloendothelial cells of the bone marrow are known to have a very low sensitivity for radiation from a morphologic point view (BLOM & JACOBSON 1948, TULLIS 1949). In addition the phagocytic function of these cells is known to be largely unaffected even by sublethal radiation doses (BARROW et coll. 1951, ANTONIJEVIC 1968, ANTONIJEVIC et coll. 1971). The radiation induced histologic changes in the bone marrow after local irradiation with small doses (MARTIN et coll. 1955), moderate to sublethal doses (FLIEDNER et coll. 1955, BOND et coll. 1962) and sublethal doses (FLIEDNER et coll. 1961, GOSWITZ et coll. 1963, KOLLATH 1963, KNOSPE et coll. 1966) are previously known. The influence of local irradiation on the function of the RES as revealed by the isotope uptake was the main purpose of the present investigation and the histologic examinations mainly served as a control of previously reported findings. Moderate (1 000 rad) to large (4 000 rad) single doses were chosen.

MALONEY & PATT (1969) *observed complete repopulation of the bone* marrow of the femur in rabbits 39 days after local irradiation with 1 000 R (300 kV) and in a later report (1972), they described variations in the hematopoietic regeneration seven months after the same dose to femur.

Most authors seem to agree that there is a stimulation of the phagocytic function on the RES during the first days after irradiation (HESS 1962, ANTONIJEVIC 1968, SLJIVIC 1970, FRED et coll. 1970, SCHILDT & ERIKSSON 1972 and others). Increased phagocytic activity was reported to occur in all dose ranges between 100 and 1 200 R (ANTONIJEVIC 1968) and between 150 and 1 600 R (HESS 1962). In the present investigation an increased uptake of colloidal ^{198}Au was found during the first two days after irradiation with 1 000 and 2 500 rad but

was observed after 2 500 rad A total depletion of hematopoietic cells occurred after 4 000 rad Rabbits were given whole body irradiation with 1 000 rad except one tib a The uptake of collo dal ¹⁹⁸Au in the irradiated tibia was not depressed After phenylhydrazine induced hemolysis increased uptake occurred in the unirradiated but not in the irradiated tibia indicating reduced proliferative capacity of the irradiated bone marrow

ZUSAMMENFASSUNG

D e Femur von Ratten wurden mit Einzeldosen von 1 000 2 500 und 4 000 rad bestrahlt D e Aufnahme von intravenos injiziertem kolloidalen ¹⁹⁸Au im bestrahlten Knochenmark wurde zu verschiedenen Zeitpunkten nach der Bestrahlung bestimmt und zur Histologie korreliert Nach 1 000 rad war die Aufnahme nur geringfugig herabgesetzt s e war starker herabgesetzt nach 2 500 rad mit Zeichen von Regeneration nach 10 Monaten Histologisch war eine Fibrose jedoch auch hamatopoietisch aktives Gewebe nach 2 500 rad zu finden Eine vollstandige Entleerung von hamatopoietischen Zellen erfolgte nach 4 000 rad Kaninchen wurde eine Ganzkorperbestrahlung mit Ausnahme einer Tib a von 1 000 rad verabfolgt Die Aufnahme von kolloidalem ¹⁹⁸Au in der bestrahlten Tib a war nicht herabgesetzt Nach phenylhydrazin hervorgerufener Hamolyse stieg die Aufnahme in der nicht bestrahlten jedoch nicht in der bestrahlten Tibia was auf eine herabgesetzte proliferative Kapazitat des bestrahlten Knochenmarks hinweist

RÉSUMÉ

Les femurs de rats ont ete irradies par des doses un ques de 1 000 2 500 et 4 000 rad La fixation dans la moelle osseuse irradiee de l or collo dal 198 injecte par voie intraveineuse a ete mesuree a differents moments apres l irradiation et a ete mise en correlation avec l h stologie Après 1 000 rad la fixation est seulement un peu diminuee et est plus marquee apres 2 500 rad avec des signes de regeneration apres 10 mois L'auteur a observe une f brose histologique mais aussi un tissu hematopoietiquement actif en voie de regeneration apres 2 500 rad Un manque total de cellules hematopoietiques apparait apres 4 000 rad Des lapins ont reçu une irradiation corporelle totale par 1 000 rad a l exception d un tibia La fixation de l or colloidal 198 dans le tibia irradie n a pas ete diminuee Apr s hemolyse indu te par la phenylhydrazine une augmentation de la fixation apparait dans le tibia non irrad e mais pas dans le tibia irradie indiquant une reduction de la capacite deproliferation de la moelle osseuse irradiee

REFERENCES

[illegible]

[illegible]

— DRAGIC M and HAJDUKOVIC A Studies on the phagocytic function of rat bone marrow after whole body irradiation I Phagocytic capability of bone marrow reticular cells in normal rats Strahlentherapie 141 (1971) 478

BARROW J TULLIS J L and CHAMBERS R W Effect of X radiation and antihistamine drugs on the reticuloendothelial system measured with colloidal radiogold Amer J Physiol 164 (1951) 822

in the unirradiated femur This is in agreement with previous histologic observations (LAWRENCE et coll 1948, GOSWITZ et coll 1963, KNOSPE et coll 1968)

After local irradiation, regeneration probably takes place through vessels growing into the injured area followed by repopulation of the bone marrow by proliferation of surviving stem cells (PATT & MALONEY 1970) or migrating hematopoietic cells (STORB et coll 1968, MALONEY & PATT 1972, TYLER & EVERETT 1972 *and others*) *Considering* these possibilities whole body irradiation was given (with the left tibia shielded) to those rabbits in which the proliferative capacity of the irradiated bone marrow was to be evaluated After total body irradiation the regeneration by newly formed vessels and migrating cells was thought to take place more slowly A local radiation injury could thus not so easily be masked by regenerative phenomena

The unirradiated RE cells proliferated rapidly after phenylhydrazine treatment as indicated by an increased uptake of ^{198}Au colloid The irradiated cells had a diminished proliferative capacity and only a small increase in the uptake A *similar depression of the proliferative capacity of the RES was reported by* WOOLES et coll (1962) Their investigations were however limited to the first 72 hours after irradiation FRED et coll (1970) found that irradiation, even with doses as high as 1 000 R, only reduced the proliferative capacity for about 7 days in whole body irradiated animals They used intravenously injected carbon particles as test substance for the of the total RES function, dominated by the function of liver and spleen and the RES was stimulutated by glucan In the present investigation a depressive action of the irradiation with 1 000 rad was observed even after 19 days The recorded differences in results may be due to *the different substances used for stimulation* of the RES The reticuloendothelial cells of the bone marrow may also behave differently compared to those of the liver and spleen Such differences have previosly been described Thus, bone marrow reticuloendothelial cells have less phagocytic capacity and react differently by phagocytosis of sequestered red cells (KEENE & JANDL 1965) Some of the present results as well as in another report (KNOSPE et coll 1968) seem to indicate that a latent injury in the slow multiplying reticuloendothelial cells of the bone marrow may be present up to about 30 days before the injury becomes evident, probably due to late cell death and impaired cell division

SUMMARY

Femurs in rats were irradiated with single doses of 1 000 2 500 and 4 000 rad The uptake of intravenously injected colloidal ^{198}Au in the irradiated bone marrow was measured at different times after irradiation and was correlated to histology After 1 000 rad the uptake was only slightly depressed and more marked after 2 500 rad with signs of regeneration after 10 months Histologically fibrosis but also regenerating hematopoietically active tissue

KOLLATH J Radiogene Schaden der Knochen, des Knochenmarks und der Gefässe nach Telekobaltbestrahlung I Mitteilung Experimente an Meerschweinchen, Bestrahlungen mit 5000 R Co[60] Strahlentherapie 123 (1963), 614

LARSSON L G and JONSSON L Totalscintigram av manniskokroppen (In Swedish) Svenska Lak-Tidn 54 (1957), 1817

— — Bone marrow scanning after intravenous injection of colloidal gold [198]Au *In* Medical radioisotope scanning, Vol II, p 193 International Atomic Energy Agency, Vienna 1964

— ENGSTEDT L, FRANZÉN S and JONSSON L Bone marrow scintigrams An in vivo study of the bone marrow reticulum in malignant bone marrow diseases Acta Un int Cancr 16 (1963), 1473

LAWRENCE J S, VALENTINE W N and DOWDY A H The effect of radiation on hemopoiesis Blood 3 (1948), 593

MALONEY M A and PATT H M Origin of repopulating cells after localized bone marrow depletion Science 165 (1969), 71

— — Migration of cells from shielded to irradiated marrow Blood 39 (1972), 804

[illegible]

irradiation of the marrow on erythron and red cell function Blood 36 (1970), 617

PATT H M and MALONEY M A Radiosensitivity of the process initiating bone marrow regeneration Radiat Res 41 (1970), 500

SCHILDT B E and ERIKSSON K H Function of the reticuloendothelial system in whole-body irradiated mice Acta radiol Ther Phys Biol 11 (1972), 48

SLJIVIC V S Radiation and phagocytic function of the reticuloendothelial system I Enhancement of RES function in X-irradiated mice Brit J exp Path 51 (1970), 130

STORB R, EPSTEIN R B and THOMAS E D Marrow repopulating ability of peripheral blood cells compared to thoracic duct cells Blood 32 (1968), 662

SATERBORG N-E Accumulation of colloidal [198]Au in normal bone marrow of rabbits Microscopic and autoradiographic investigation Acta radiol Ther Phys Biol 13 (1974), 205

TAVASSOLI M and WEISS L The structure of developing bone marrow sinuses in extramedullary autotransplant of the marrow in rats Anat Rec 171 (1971), 477

TULLIS J L The response of tissue to total body irradiation Amer J Pathol 25 (1949), 829

TYLER R W and EVERETT N B Radioautographic study of cellular migration using parabiotic rats Blood 39 (1972), 249

WOOLES W R, ELKO E E and DI LUZIO N R Influence of pre- and post- X irradiation zymosan administration on reticuloendothelial function Radiat Res 16 (1962), 546

Bloom W and Jacobson L O Some hematologic effects of irradiation Blood 3 (1948) 586

Bond V P Fliedner T M and Usenik E Early bone marrow hemorrhage in the irradiated dog Arch Path 73 (1962) 13

Brånemark P I Breine U Johansson B Roylance P J Hockert H and Yoffey J M Regeneration of bone marrow A clinical and experimental study following removal of bone marrow by curettage Acta anat (Basel) 59 (1964) 1

Caffrey R W Everett N B and Rifke W O Radioautographic studies of reticular and blast cells in the hematopoietic tissues of the rat Anat Rec 155 (1966) 41

Edwards C L Andrews G A Sitterson B W and Kniseley R M Clinical bone marrow scanning with radioisotopes Blood 23 (1964) 741

Engstedt L Franzén S Jonsson L and Larsson L G *In vivo localization of colloidal* ^{198}Au intravenously injected in polycythemia vera Acta radiol 49 (1958) 66

Flemming K Flemming C and Nothdurft W The phagocytic activity of the reticuloendothelial system of mice following whole body X irradiation J reticuloendothelial Soc 7 (1970) 1

Fliedner T M Sandkühler S und Stodtmeister R Die Knochenmarkstruktur bei Ratten nach Bestrahlung mit schnellen Elektronen Z Zellforsch 43 (1955) 195

— Bond V P and Cronkite E P Structural cytologic and autoradiographic (H^3 Thymidine) changes in the bone marrow following total body irradiation Amer J Pathol 38 (1961) 599

Fred K Dobson F L Kelly L S and Shore M L The proliferative and phagocytic capacity of the reticuloendothelial system of sublethally irradiated mice *J reticuloendothelial Soc* 7 (1970) 453

Goswitz F A Andrews G A and Kniseley R M Effects of local irradiation (Co^{60} teletherapy) on the peripheral blood and bone marrow Blood 21 (1963) 605

Haas R J Meyer Hamme K Fliedner T M Barthel E Fache I and Steinhoff K The role of transplanted slowly proliferating bone marrow cells for regeneration of lethally X irradiated rat bone marrow Scand J Haemat 9 (1972) 121

Harris P F Haigh G and Kugler J H Quantitative studies of mitoses and DNA synthesizing cells in bone marrow and blood of guinea pigs recovering from sublethal whole body gamma irradiation Brit J Haemat 9 (1963) 385

Hess F Die Dynamik des retikuloendothelialen Systems nach einem Strahleninsult Strahlentherapie *119 (1962) 161*

International commision on radiological units and measurements (ICRU) Report No 10 d Clinical dosimetry National Bureau of Standards Handbook 87 NBS Appendix II Dosage in bone p 45 Washington 1963

Keene W R and Jandl J H Studies of the reticuloendothelial mass and sequestering function of rat bone marrow Blood 26 (1965) 157

Kjellgren O and Jonsson L Bone marrow depression in the pelvis after megavoltage irradiation for ovarian cancer Amer J Obstet Gynec 105 (1969) 849

Kniseley R M Andrews G A Edwards C L and Tandida R Scanning of bone marrow in hematopoietic disorders *In* Medical radioisotope scanning Vol II p 207 International Atomic Energy Agency Vienna 1964

Knospe W H Blom J and Crosby W H Regeneration of locally irradiated bone marrow I Dose dependent long term changes in the rat with particular emphasis upon vascular and stromal reaction Blood 28 (1966) 398

— — — Regeneration of locally irradiated bone marrow II Induction of regeneration on permanently aplastic medullary *cavities Blood 31 (1968) 400*

KOLLATH J Radiogene Schaden der Knochen, des Knochenmarks und der Gefasse nach Telekobaltbestrahlung I Mitteilung Experimente an Meerschweinchen, Bestrahlungen mit 5000 R Co^{60} Strahlentherapie 123 (1963), 614

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MALONEY M A and PATT H M Origin of repopulating cells after localized bone marrow depletion Science 165 (1969), 71

— — Migration of cells from shielded to irradiated marrow Blood 39 (1972), 804

MARTIN I, PAPE R und PIRINGER KUCHINSKA A Zur Frage der Wirkung kleinster Röntgen dosen auf das Knochenmark Strahlentherapie 96 (1955), 569

NELP W B, GOHIL M N, LARSSON S M and BOWER R E Long term effects of local irradiation of the marrow on erythron and red cell function Blood 36 (1970), 617

PATT H M and MALONEY M A Radiosensitivity of the process initiating bone marrow regeneration Radiat Res 41 (1970), 500

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SÄTERBORG N E Accumulation of colloidal ^{198}Au in normal bone marrow of rabbits. Microscopic and autoradiographic investigation Acta radiol Ther Phys Biol 13 (1974), 205

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TYLER R W and EVERETT N B Radioautographic study of cellular migration using parabiotic rats Blood 39 (1972), 249

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FROM THE SECTION OF EXPERIMENTAL RADIATION THERAPY, UNIVERSITY OF VIRGINIA SCHOOL OF MEDICINE, CHARLOTTESVILLE, VIRGINIA 229 01 USA

RADIATION RESPONSE OF MURINE ADENOCARCINOMA AS A FUNCTION OF SERIAL TRANSPLANTATION

A M EL-MAHDI, J SHAEFFER and W C CONSTABLE

Serial transplantation of animal tumors can lead to different changes in the biologic characteristics of the tumor MORIWAKI et coll (1971) have shown that ploidy fluctuates in the MPCN-1 tumor as a result of serial transplantation Changes in the histologic appearances of transplanted murine tumors in the direction of increased cellular anaplasia have also been reported to occur as a result of successive transplantation, these structural alterations may occur as early as the first generation or they may develop gradually following several generations (STEWART et coll 1959)

Alterations of radiation response as a result of serial transplantation has not been thoroughly investigated CD2F$_1$ murine mammary adenocarcinoma both in vivo and in vitro in its early (6—12th) generations is highly radiation sensitive (SHAEFFER et coll 1972) Following continued transplantation, the tumor dramatically changed its in vivo radiation response In an attempt to shed some light on the cellular nature of this shift in the in vivo sensitivity, a cell line was cultured from the resistant tumor In vitro survival curves and karyotypes were determined on the resistant-derived cultured line Changes in both in vivo and in vitro sensitivity, as well as ploidy fluctuations, are discussed as a function of serial transplantation

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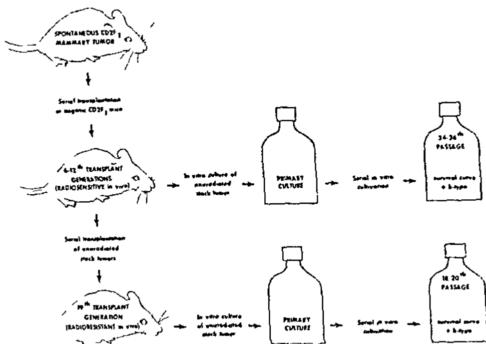


Fig 1 Schematic history of the CD2F1 mammary adenocarcinoma

Material and Methods

Mice CD2F1 females, aged 9 to 12 weeks, were obtained from the Cumberland View Farm, Clinton, Tennessee They were housed in covered plastic cages, 4 to 8 per cage, and provided with a standard laboratory diet and tap water ad libitum

In vivo sensitivity Tumor cell suspensions were prepared in normal saline at a concentration of 1×10^7 cells/ml Mice were injected in the lateral aspect of the right hind leg with 2×10^6 cells in 0 2 ml Irradiation was begun 8 to 10 days after injection, by which time the average tumor diameters were approximately 2 cm In vivo irradiations utilized a therapy unit (140 kV, 18 mA, HVL 6 mm Al, TSD 40 cm) The dose distribution under these conditions was 92 rad/min at the skin surface, 75 rad/min at a depth of 1 cm, and 60 rad/min at a depth of 2 cm All doses were calculated at a depth of 1 cm, which corresponded to the center of the tumor Mice were kept in restraining devices without anesthesia during irradiation The mice were completely shielded except for the tumor-bearing regions which were treated by a single field Tumor diameters were measured by calipers throughout the course of the experiments

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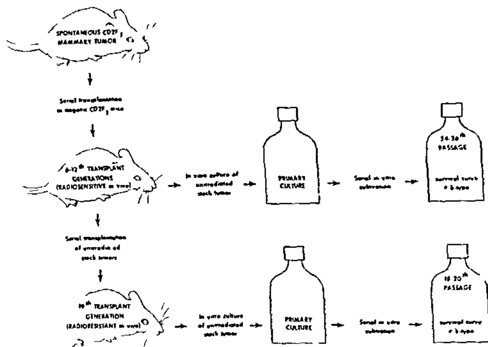


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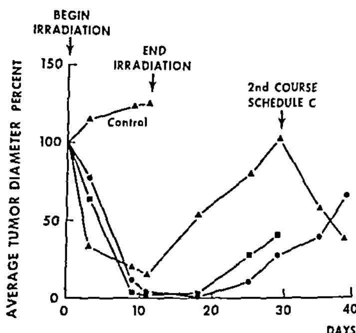


Fig 2 In vivo radiation responses of CD2F1 mammary adenocarcinoma in its early (6—12th) generations Controls were unirradiated Dose fractionation schedules A B and C are explained in the text Each point represents an average tumor diameter computed from 6 mice

In vitro cell survival Cell lines were initiated and maintained as previously described (SHAEFFER et coll 1972) For in vitro cell survival experiments, appropriate numbers of cells from exponentially growing stock cultures were seeded onto 15 × 100 mm glass Petri plates Before irradiation, the seeded plates were put in a humidified 10 % CO_2 incubator for two hours In vitro irradiations utilized a constant potential therapy machine (250 kV, 15 mA, HVL 0 52 mm Cu) The cultures were irradiated at room temperature at an exposure rate of 200 R per minute Orcein stained colonies were counted 10 to 14 days after irradiation Per cent survivals and standard deviations were computed from colony counts on 3 replicate plates at each dose point

Karyotypic determinations Cultures grown on glass slides were given vincristin sulfate (Eli Lilly Co) at a final concentration of 1×10^{-6} per cent for 3 hours to collect metaphase cells The medium was replaced with distilled water at 37° C for 10 minutes to induce cell swelling, following this, the cells were fixed in 1 3 acetic methanol, stained with Giemsa, and mounted

Results

A schematic history of the $CD2F_1$ mammary tumor is presented in Fig 1 In vivo radiation responses of the tumor in the 6th to 12th generation from the spontaneous tumor are shown in Fig 2 The tumor demonstrated a high degree

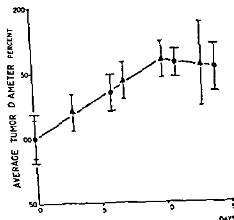


Fig 3 In vivo radiation response of the CD2F1 mammary adenocarcinoma (19th generation) Controls were unirradiated The irradiated mice (schedule D) were given 10 fractions of 200 rad/fraction in an overall time of 14 days for a cumulative total of 2 000 rad Each point represents an average tumor diameter computed from 40 mice bars represent the standard deviation ● irradiated ▲ controls

of sensitivity under a variety of fractionation schedules Schedule A consisted of 9 fractions of 200 rad per fraction in an overall time of 12 days for a total 1 800 rad In schedule B, a 500 rad conditioning dose was followed by 5 fractions of 180 rad each and 3 fractions of 160 rad each in an overall time of 12 days for a cumulative total dose of 1 880 rad In schedule C, a 1 000 rad conditioning dose was given, followed by 5 fractions of 143 rad each and 2 fractions of 103 rad each in an overall time of 11 days for a cumulative total dose of 1 921 rad In schedule C, after the tumors had regrown to 103 per cent of their original diameters (29th day), a second course of two 572 rad treatments was started to test the radiation response of the recurrent tumors The following results were obtained (1) high radiation response of the early tumor under different fractionation schedules as manifest by rapid decrease in tumor diameter, (2) rapid local recurrence of the tumor after the initial response, and (3) the retained radiation sensitivity of the recurrent tumors

The results of the tumor response to irradiation at its 19th generation from the spontaneous tumor are given in Fig 3 Ten fractions of 200 rad each in an overal ltime of 14 days for a total dose of 2 000 rad were used The irradiated tumors did not only lack regression, but they grew at a rate not significantly different from that of unirradiated controls It is also apparent from Fig 3 that the unirradiated (control) tumors at the 19th transplant generation were growing at a faster rate than the control tumors in the 6th to 12th generations (Fig 2)

The in vitro survival curve and karyotype of a cell line cultured from the sensitive (12th generation) tumor (Fig 1) have been previously reported (SHAEFFER et coll 1972) In the present investigation, the in vitro survival curves and karyotypes were determined on a cell line cultured from the more

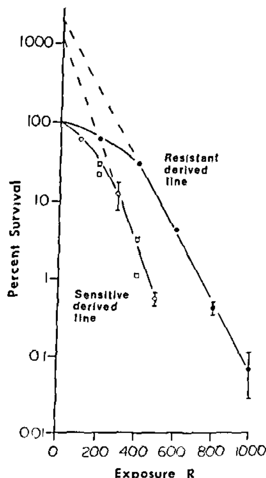


Fig 4 In vitro survival curves of the cell lines cultured from early (sensitive) and late (relatively resistant) mouse mammary adenocarcinomas Standard errors are designated by bars □ — average per cent survivals from 3 replicate plates per dose point in the cell line derived from the relatively resistant adenocarcinoma ● — average per cent survivals from 3 replicate plates per dose point in the cell line derived from the sensitive adenocarcinoma ○ — average *per cent survivals from 2 replicate plates per* dose point in a second experiment using the sensitive derived line

resistant (19th generation) tumor (Fig 1) Both lines were relatively radiation sensitive (Fig 4), the sensitive-derived line having a D_0 of 65 R compared to a value of 92 R for the resistant-derived line The most striking difference in the survival curves occurred in the shoulder region The sensitive-derived line had an extrapolation number of 12 8 with a D_0 of 166 R while the values for resistant derived line were 18 3 and 267, respectively Both lines appeared as epithelioid cells and had comparable doubling times 27 hours for the sensitive-derived line and 23 hours for the resistant derived line

Representative karyotypes of both cell lines are presented in Figs 5 and 6 and the chromosome frequency distributions in the Table The sensitive-derived line was near tetraploid, having a mean chromosome number of 82 and a mode of 86 Two types of marker chromosomes were present (Fig 5) The first was an elongated acrocentric which was present in all 50 metaphase plates scored, the second marker chromosome was a submetacentric chromosome Of all 50 cells

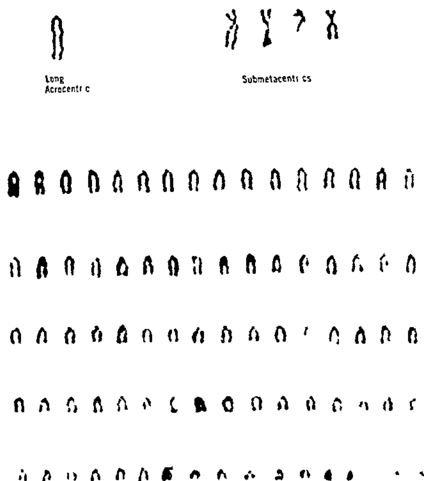


Fig 5 Representative karyotype of the cultured CD2F1 line derived from the sensitive 12th transplant generation The marker chromosomes a long acrocentric and 4 submetacentric chromosomes are grouped at the top This metaphase cell contained a total of 86 chromosomes The average number of chromosomes in 50 metaphase cells was 82

scored, 44 contained 4 of the submetacentric chromosomes, 5 cells contained 3 submetacentric chromosomes, and the remaining cell had but two submetacentrics



Fig 6 Representative karyotype of the cultured CD2F1 line derived from the resistant 19th transplant generation The marker chromosomes 2 long acrocentric and 8 submetacentric chromosomes are grouped at the top This metaphase cell contained a total of 108 chromosomes The average number of chromosomes in 50 metaphase cells was 95

The resistant derived line had a mean chromosome number of 95, with a mode of 80 As illustrated in Fig 6, more marker chromosomes were present An average of 1 2 elongated acrocentrics per cell (as opposed to an average of 1 0

Table

Chromosome frequencies of cultured $CD2F_1$ lines

A Sensitive-derived line from 12th transplant generation										
Chromosomes	53	70	75	76	77	78	79	80	81	
Cells	1	1	1	1	1	3	1	7	6	
Chromosomes	82	83	84	85	86	87	88	90	94	
Cells	5	2	4	2	8	3	1	1	2	
B Resistant-derived line from 19th transplant generation										
Chromosomes	69	72	73	76	79	80	82	83	84	
Cells	1	1	1	1	1	7	2	3	5	
Chromosomes	85	86	87	88	89	92	95	96	103	106
Cells	4	2	1	2	2	2	2	2	1	1
Chromosomes	112	120	122	132	133	135	162	163	187	
Cells	1	1	1	1	1	1	1	1	1	

for the sensitive derived line) and an average of 5 6 submetacentrics per cell (as opposed to an average of 3 9 for the sensitive-derived line) were observed,

Cell lines cultured from both the 12th and 19th generations were tested for their tumorigenic capacities by injecting cultured cells (5×10 cells/mouse) subcutaneously into the flanks of 34 mice Within 2 weeks, there was a 100 per cent incidence of palpable tumors in both groups of injected mice, demonstrating that both cultured lines had retained their capacity for inducing tumors in vivo These tumors have been pathologically confirmed as adenocarcinomas

Discussion

The results point out a remarkable difference in tumor response between early and later generation tumors The question naturally arises as how to explain this difference in the in vivo response on the basis of the survival curves obtained in vitro Although these in vitro survival curves do reveal similar D_0 they have dramatically different shoulders, $D_q = 166$ R for the sensitive-derived line and $D_q = 267$ R for the resistant-derived line The calculated values of D_{10} for the sensitive and resistant-derived lines were 315 and 505 R, respectively This broadening of the shoulder width in the resistant-derived line may indicate an increase in the capacity for sublethal repair in this cell line

In the in vivo experiments, the radiation dose was delivered in a fractionated scheme, compared to the single doses employed in the in vitro experiments. If the results of the in vitro curves are utilized to explain the cumulative repair in the

in vivo experiments, the decreased radiation response of the more resistant tumor may be explained, at least in part, on the basis of its increased capacity for repair (HORNSEY 1972)

The main changes in the karyotypic profiles of the two lines were that the resistant line had a higher mean chromosomal number and more marker chromosomes SPARROW & EVANS (1961) have suggested that, in general, increased chromosome number in a given cell line will decrease cellular radiation sensitivity Although our karyotype findings are generally in agreement with the hypothesis of SPARROW & EVANS, other workers have shown either no relationship (TILL 1961) or a questionable relationship (LOCKHART et coll 1961) between the chromosome number and radiation sensitivity The change in marker chromosomes was primarily in the submetacentric chromosomes, where there were an average of 3 9 in the sensitive-derived line compared to 5 6 in the resistant-derived line The genetic consequences of such changes in marker chromosomes might be manifested by an alteration in the magnitude of radiation insult or the degree of repair or both

It might be expected that the sensitive tumor would exhibit a higher degree of anaplasticity and mitotic figures than the resistant tumor (RUBIN & CASARETT 1968) It is of interest that there was no change in the histologic appearances between the radiation resistant and the sensitive tumor Both tumors were highly undifferentiated adenocarcinomas (SHAEFFER & EL-MAHDI 1973)

If a tumor is composed of a heterogeneous cell population in regard to radiation response, it may be assumed that irradiating the sensitive tumor would eradicate the sensitive cell population, with minimal effect on the more resistant cell capable of initiating tumor recurrence The present results demonstrate that irradiating the sensitive tumor brought about immediate tumor regression followed by rapid local recurrence (Fig 2), but a second course of irradiation revealed the recurrent tumors to be as sensitive as the primary ones (Fig 2 c) Such results cannot explain the change in the in vivo sensitivity based solely on heterogeneity of the tumor population and natural selectivity of the relatively resistant cells after serial transplantation

In conclusion, the results have shown that serial transplantation of animal tumors have led to a change in radiation response This is an observation which should be kept in mind when evaluating results using animal tumors

SUMMARY

Fractionated doses of approximately 2 000 rad resulted in remarkable tumor shrinkage using early generation transplants of murine mammary *adenocarcinoma A cell line cultured* from unirradiated stock tumors of this early generation transplant had a D_1 of 65 R Karyotypically the line was aneuploid having a mean chromosome number of 82 and several

marker chromosomes Following continued transplantation the tumor became highly radiation resistant when irradiated in vivo A cell line cultured from unirradiated stock tumors of this late generation transplant had a D_0 of 92 R and a slightly increased mean chromosome number (95)

ZUSAMMENFASSUNG

Fraktionierte Dosen von etwa 2000 rad fuhrten zu einer ausgezeichneten Tumor schrumpfung wenn Transplantete junger Generation muriner Mamma Adenokarzinome ver wendet wurden Eine Zell Linie, die von einem nicht bestrahlten Stammtumoren des Trans- plantats dieser jungen Generation kultiviert worden war hatte eine D_0 von 65 R In ihrem Karyotyp war diese Linie aneuploid mit einer mittleren Chromosomennummer von 82 und verschiedenen Markor Chromosomen Nach einer langer anhaltenden Transplantation wurde der Tumor bei Bestrahlung in vivo stark strahlenresistent Eine von dem unbestrahlten Stammtumor kultivierte Zell Linie vom Transplantat dieser alten Generation hatte eine D_0 von 92 R und eine leicht erhohte mittlere Chromosomenzahl (95)

RÉSUMÉ

Des doses fractionnees d environ 2000 rad ont produit une diminution de volume re- marquable de la tumeur provenant de transplants de generations precoces d adenocarcinome mammaire de la souris Une lignee *de cellules cultivees* à partir de tumeurs souches non irradiees de cette transplantation de generations precoces avait une D_0 de 65 R Au point de vue caryotype que cette lignee etait aneuploide ayant un nombre moyen *de chromosomes de* 82 et plusieurs chromosomes traceurs Apres poursuite de la transplantation la tumeur est devenue tres radioresistante quand elle etait irradiee in vivo Une lignee cellulaire cultivee à partir de tumeurs souches *non irradiees de ce transplant* de generations tardives avait une D_0 de 92 R et un nombre moyen de chromosomes legèrement augmente (95)

REFERENCES

HORNSEY S The radiosensitivity of melanoma cells in culture Brit J Radiol 45 (1972) 158

LOCKHART L Z, ELKIND M M and MOSES W B Radiation response of mammalian cells [illegible]

Mc [illegible] plasma cell neoplasm MSPC-1 during serial transplantation J nat Cancer Inst 47 (1971), 623

RUBIN P and CASARETT G W Clinical radiation pathology W B Saunders Philadelphia 1968

SHAEFFER J and EL MAHDI A M Another look at the law of Bergonié and Tribondeau Beitr Path 149 (1973), 408

— and MERZ T In vivo and in vitro radiation responses of a sensitive spontaneous mouse mammary adenocarcinoma Cancer 29 (1972), 581

SPARROW A H and EVANS H J Nuclear factors affecting radiosensitivity I The influence of nuclear size and structure, chromosome complement, and DNA content *In* Fundamental aspects of radiosensitivity Vol 14, p 76 Brookhaven Symposia in Biology, Upton 1961

STEWART H L, SNELL K C, DUNHAM L J and SCHLYEN S M Transplantable and transmissable tumors of animals Vol F-40, p 20 Armed Forces Institute of Pathology Washington 1959

TILL J E Radiosensitivity and chromosome numbers in strain L mouse cells Radiat Res 15 (1961), 400

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J BERGSTROM and C O HENRIKSON

Periodontal surgery implies a trauma to the supporting periodontium of the teeth Little is known about the reaction of the alveolar bone to this insult Histology indicates, however, that an initial resorption occurs which later may be followed by the formation of new bone (WILDERMAN 1963) Any permanent loss of alveolar bone after conventional methods of periodontal surgery—gingivectomy and mucoperiosteal flap operation—is considered 'small' (FRIEDMAN & LEVINE 1964) However, the extent of bone loss and the degree of subsequent repair is not known in quantitative terms

One way quantitatively to express alterations in bone tissue is to calculate the amount of mineral mass per unit area of the bone in question (OMNELL 1957) The principles for the measurement of changes of the mineral content of jawbone in vivo by means of radiation from ^{125}I have been presented by HENRIKSON (1967) The technique was utilized by BERGSTROM & HENRIKSON (1970) for

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Table 1

Patient data for the present material Bone height was measured on intraoral films in per cent of the distance apex — cementoenamel junction of the lateral incisor F = flap operation, G = gingivectomy

Case No	Sex	Age	Bone height in per cent of root length		Interproximal pocket depth (mm) of region		Surgical procedure	
			Right	Left	Right	Left	Right	Left
1	M	42	86 7	85 7	4 5	5 0	F	G
2	M	41	68 8	70 6	4 5	4 5	F	G
3	M	42	72 5		5 0		G	
4	M	47		73 3		5 0		G
5	M	39	88 2		5 5		G	
6	F	41	76 5	76 5	5 5	5 0	F	G
7	F	54	76 5	73 3	4 5	5 0	F	G
8	F	47	85 7	71 4	5 0	7 0	F	G
9	F	42	73 7	68 4	4 0	6 5	F	G
10	M	49	70 6	85 7	4 5	4 0	F	F
11	M	49		70 6		5 0		F
Mean		44 8	77 68	75 05	4 78	5 22		

evaluating changes in interdental alveolar bone following mucoperiosteal flap surgery The mineral content was expressed in aluminium equivalents and the measurements gave evidence of demineralization of the interdental bone from about two weeks after the operation, minimum values were observed after 3 to 6 weeks, after which a continuous increase throughout the observation period usually followed

However, changes in volume of the soft tissue probably affected the evaluation of the changes in bone mass, especially during the first two weeks after the operation The method was therefore improved in order to make possible measurements of the thickness of the alveolar process in the recorded area (HENRIKSON & JULIN 1971) With a knowledge of the thickness of the alveolar process in the direction of the radiation beam the influence from fluctuations of tissue volume on total transmission will be kept under control and the amount of bone mineral can be determined in terms of mass per unit of area, e g mg mm⁻²

The purpose of the present report is quantitatively to describe the changes in mineral mass of the interdental alveolar bone in connection with surgery of patients with periodontal disease The postoperative swelling in the same region was recorded as well

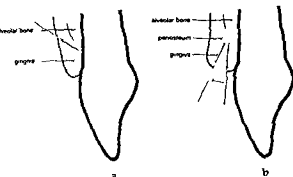


Fig 1 a) The gingivectomy technique b) The mucoperiosteal flap procedure

The material consists of 11 patients, 4 female and 7 male, referred to the Department of Periodontology, for treatment of periodontal disease Their periodontal condition, though not advanced to such a degree as the tooth mobility was notably increased, indicated surgery Data for the total material are given in Table 1 All patients had a sufficient number of teeth in the upper jaw to guarantee necessary stability of the measurement apparatus

Before any measurements, dental plaque and calculus were removed The patients were also instructed in oral hygiene and informed of its importance Only those who cooperated in this respect were included in the material The oral hygiene of the patients was continuously supervised during the observation period The patients were observed during a six month period and observations were made mainly according to the following schedule on 2 to 3 occasions before operation with at least one week's interval, at weekly intervals during the first postoperative month, every second week during the next two months, and every third week during the remaining three months of the observation period

Methods

Surgical procedure Two types of periodontal surgery were used in nine instances a gingivectomy was performed and in nine instances a mucoperiosteal flap operation Most patients were operated on by gingivectomy in one area and by flap operation in the contralateral (Table 1) Both methods are conventional modalities of surgery for the elimination of periodontal pockets, the principal difference being that the flap procedure involves temporary bone exposure while the gingivectomy does not

In areas where gingivectomy was performed the diseased gingiva labially, lingually and interdentally was eliminated The surface of the subjacent marginal alveolar bone was not exposed but was covered by a remaining layer of soft tissue The treated area was covered with a surgical dressing for one week (Fig 1 a)

Table 1

Patient data for the present material Bone height was measured on intraoral films in per cent of the distance apex — cementoenamel junction of the lateral incisor F = flap operation, G = gingivectomy

Case No	Sex	Age	Bone height in per cent of root length		Interproximal pocket depth (mm) of region		Surgical procedure	
			Right	Left	Right	Left	Right	Left
1	M	42	86.7	85.7	4.5	5.0	F	G
2	M	41	58.8	70.6	4.5	4.5	F	G
3	M	42	72.5		5.0		G	
4	M	47		73.3		5.0		G
5	M	39	88.2		5.5		G	
6	F	41	76.5	76.5	5.5	5.0	F	G
7	F	54	76.5	73.3	4.5	5.0	F	G
8	F	47	85.7	71.4	5.0	7.0	F	G
9	F	42	73.7	68.4	4.0	6.5	F	G
10	M	49	70.6	85.7	4.5	4.0	F	F
11	M	49		70.6		5.0		F
Mean		44.8	77.68	75.05	4.78	5.22		

evaluating changes in interdental alveolar bone following mucoperiosteal flap surgery The mineral content was expressed in aluminium equivalents and the measurements gave evidence of demineralization of the interdental bone from about two weeks after the operation, minimum values were observed after 3 to 6 weeks, after which a continuous increase throughout the observation period usually followed

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The purpose of the present report is quantitatively to describe the changes in mineral mass of the interdental alveolar bone in connection with surgery of patients with periodontal disease The postoperative swelling in the same region was recorded as well

Table 2

Values of bone mineral mass in mg mm⁻² for regions treated by flap operation The preoperative value (0) is the mean of 2 to 3 observations before the operation

Case No	Week 0	1	2	3–4	6–8	10–12	14–16	18–20	24–26
1	4 16	4 20	4 14	4 0?	4 00	4 15	4 00	4 02	4 01
2	4 37	3 88	3 66	3 72	3 80	4 02	4 08	4 14	4 16
7	4 50	4 42	4 25	4 19	4 15	4 13	4 32	4 37	4 29
11	2 09	2 04	1 62	1 74	1 90	1 82	2 48	(2 49)*	2 50
6	4 15	4 16	3 58	3 27	3 48	3 79	3 81	3 98	4 09
8	3 17	3 07	2 73	2 81	3 04	3 13	(3 18)*	(3 18)*	3 22
10	3 91	3 92	3 77	3 66	3 78	3 67	(3 82)*	3 97	(3 97)*
10	0 56	0 60	0 37	0 37	0 42	0 38	0 40	0 41	0 39
9	4 47	4 28	4 23	4 10	4 12	4 08	4 38	4 53	4 58
M	3 4866	3 3966	3 1500	3 0977	3 1877	3 2411	3 3855	3 4544	3 4677
SD	1 3445	1 2909	1 3431	1 2819	1 2553	1 3052	1 2681	1 3038	1,3132
SE	0 4481	0 4303	0 4477	0 4273	0 4184	0 4350	0 4227	0 4346	0 4377
N	9	9	9	9	9	9	(9)	(9)	(9)

* Value found by inter or extrapolation

$$s_d = \sqrt{\frac{\sum d_i^2}{2n}}, \text{ where}$$

d = difference between two replicate determinations

n = number of differences

The mean value of the precision for the 18 regions examined was 0 038 mg mm⁻², the range being 0 02 to 0 06 mg mm⁻² (Fig 2) No significant differences were noted as regards the mean precision value for the first four postoperative weeks as compared to that of the subsequent observation periods, 6 to 12 weeks and 14 to 26 weeks, or to the preoperative mean precision value

The thickness of the alveolar process has been determined separately in connection with the transmission measurements. The mean precision of the thickness determinations as expressed by the standard deviation of repeated settings and readings throughout the observation period was 0 13 mm for the total material

Results

Changes in interdental bone mineral mass The values of the bone mineral mass for the present material during the period of observation are given in Tables 2 and 3 As a mean for all areas treated the preoperative value of bone

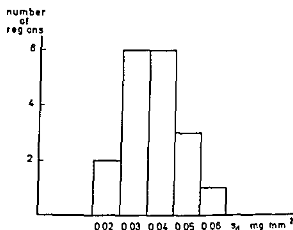


Fig 2 Distribution of precision values, s_1 for eighteen regions

The mucoperiosteal flap method implied a deflection by blunt dissection of the labial gingiva in the area This was accomplished by an internal bevelled incision along the gingival margin and from the margin down to the crest of the alveolar bone, followed by elevation of a flap containing mucosa and periosteum (Fig 1 b) Lingual pockets were removed by curettage The epithelial lining of the pocket wall as well as granulation tissue and subgingival plaque were removed No correction of the alveolar bone was made The flap was replaced and sutured interdentally and the treated area was covered with surgical dressing for at least one week (For a more detailed explanation of the surgical techniques the reader is referred to standard textbooks, e g GLICKMAN 1972, GOLDMAN & COHEN 1968)

Recording procedure With the apparatus used it is possible to measure the transmission of [1]· I radiation through a certain part of the alveolar process and also to measure the thickness of the same part of the alveolar process The apparatus and the measurement technique have been described by HENRIKSON & JULIN (1971) and HENRIKSON & BERGSTROM (1974) The measurement of bone mineral mass and thickness refer to the interdental region between a canine and a lateral incisor or between a medial and a lateral incisor of the upper jaw

The transmission was measured in two positions, the interdistance between such measurement points being 0 3 to 0 5 mm The position of a measurement point in relation to adjacent teeth was controlled on a series of roentgenograms of the area by projection of steel balls (1 mm in diameter) at the points of entrance and exit of the radiation beam on lingual and labial gingiva

All values of mineral mass obtained were corrected for the 'shorter wave length' of the radiation (HENRIKSON & BERGSTROM 1974)

The precision of the method s_1, was determined on the basis of replicate determinations according to the formula

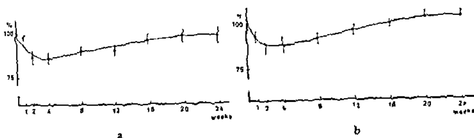


Fig 3 Percentual changes with time in relation to preoperative values of bone mineral mass Mean and SE for a) nine regions treated with flap operation and b) nine regions operated upon with gingivectomy

Changes in the thickness of the alveolar process in relation to the preoperative level during the period of observation are given in Fig 4 as averages for the two groups It is evident from the graph that the thickness increased immediately after operation in both groups and more so for the flap operation group At the one week postoperative observation the mean increase was 1 24 ± 0 21 mm (M ± SE) for the flap operations and 0 66 ± 0 17 mm (M ± SE) for the gingivectomies The changes are statistically significant ($t = 5.91$ and 3 88, respectively, $p < 0.01$)

During the subsequent period of observation this increase declines towards the preoperative level in both groups As for the gingivectomy group the preoperative value is reattained after two to four weeks and from about eight weeks after operation the alveolar thickness is less than preoperatively, significantly so at the end of the observation period ($t = 2.82$, $p < 0.05$)

The subsiding of the postoperative swelling is slower in the flap operated areas and a return to the preoperative level does not occur completely during the period of observation

Discussion

The general course of the postsurgical reaction of the alveolar bone for both groups of treated areas is rather uniform, characterized by an initial loss of alveolar bone mass followed by a gradual recovery At the end of the observation period the alveolar bone mass approximates the preoperative level This course of events gives further support to earlier findings on a similar patient material (BERGSTROM & HENRIKSON 1970) when the changes of the alveolar process were expressed in aluminium equivalents Converting these latter values to mg mm^{-2} hydroxyapatite, it will be seen that for both of these materials, altogether 25 treated regions, the magnitude of the initial decrease was approximately 0 3 mg mm^{-2}

Table 3

Values of bone mineral mass in mg mm⁻² for regions treated by gingivectomy The 0 value represents the mean of 2 to 3 observations before the operation

Case No	Week								
	0	1	2	3–4	6–8	10–12	14–16	18–20	24–26
1	4.48	4.48	4.25	4.18	4.38	4.64	4.60	4.55	4.54
2	4.20	4.03	4.06	3.75	3.85	4.25	4.61	4.66	4.48
7	2.69	2.53	2.31	2.47	2.47	2.58	2.63	2.50	2.56
4	4.45	3.98	3.69	3.86	3.83	4.28	4.46	4.50	4.43
6	4.00	3.63	3.72	3.65	3.78	3.87	3.88	3.90	4.07
5	1.62	1.15	0.83	0.90	1.18	1.22	1.50	1.64	(1.57)*
3	3.98	3.97	3.99	3.95	3.97	4.00	(4.06)*	(4.06)*	4.12
8	2.30	2.28	2.11	2.05	2.06	(2.17)*	(2.17)*	2.27	2.42
9	3.59	3.50	3.62	3.59	3.65	(3.74)*	3.82	4.48	4.43
M	3.4788	3.2833	3.1755	3.1555	3.2411	3.4166	3.5255	3.6177	3.6244
SD	1.0288	1.0746	1.1589	1.1035	1.0747	1.1546	1.1425	1.1581	1.1245
SE	0.3429	0.3582	0.3863	0.3678	0.3582	0.3848	0.3806	0.3860	0.3748
N	9	9	9	9	9	(9)	(9)	(9)	(9)

* Value found by inter- or extrapolation

mineral mass was 3.48 mg mm⁻² (3.487 and 3.479 mg mm⁻² in flap and gingivectomy areas, respectively) and the range was 0.56 to 4.47 mg mm⁻²

The response of the alveolar bone to the surgery as reflected by the values of mineral mass is similar in the two groups It is characterized by an initial decrease, most marked 3 to 4 weeks after the operation Intraindividual differences were determined between the alveolar bone mass value obtained before and 3 to 4 weeks after surgery The mean value of these differences was 0.389 ± 0.078 mg mm⁻² (M ± SE) and 0.322 ± 0.079 mg mm⁻² (M ± SE) for the flap operation and gingivectomy areas, respectively These mean differences are significant ($t = 4.99$ and 4.06, respectively, $p < 0.01$)

The values of mineral mass during the period of observation expressed in per cent of the preoperative value are given in Fig 3 The average decrease at the three to four week observation was then 13.67 and 11.52 per cent for the flap operation and gingivectomy areas, respectively These percentage changes are statistically significant ($t = 4.33$ and 2.64, respectively, $p < 0.01$)

After the initial period of decreasing mineral mass increasing values were again encountered in both types of treatment For the total material the preoperative level is reestablished after about 16 to 18 weeks The process of return is somewhat faster in the gingivectomy treated areas

mass analogous to that of WILDERMAN (1963), it seems appropriate to call the initial reaction, 0 to 30 days, the demineralization stage (corresponding to the preparatory, osteoclastic and osteoblastic stages of WILDERMAN) and consequently the period 30 to 180 days after the injury the remineralization stage (corresponding to the maturation stage)

The quantitative analysis of mineral mass changes of the alveolar bone reported was performed in its marginal interdental part The height of the remaining margin was reduced by, on an average, 15 per cent compared to normal, which is generally regarded to be about 90 per cent of the root length (Table 1)

These regions varied in mineral mass within rather wide limits (0 56 to 4 48 mg mm^{-1}) Such interindividual variation may in part be qualitative but may also represent the orientation of the radiation beam within the subject on passage through the alveolar process, and particularly its relation to the alveolar margin

It has been reported (WILDERMAN et coll 1960, WILDERMAN 1963, WOOD et coll 1972, TAVTIGIAN 1970) that the margin is especially sensitive to resorptive changes after surgery In the present material no significant correlation was found between the presurgical amount of the alveolar bone and the severity of the initial reaction (expressed as loss of mineral mass) Further measurements, however, should be made to evaluate the magnitude of such changes for bone layers at different distance from the margin

On the other hand there was rather close conformity of the type of reaction between the two measurement positions within a certain region and at the given height level This indicates that possible erroneous influences due to a contribution to attenuation from dentine and cementum of adjacent teeth were limited, if at all present As the apparatus was constructed also for film exposure by the extremely collimated radiation, the tissue structure at the positions selected for recording could be examined Bone structure was demonstrated in the films of all areas

As was demonstrated by FORSBERG & HAGGLUND (1957) the trauma from periodontal surgery will result in an at least temporarily increased mobility of the teeth The increased tooth mobility may affect the reliability of the value of mineral mass obtained during the first postoperative period However, judging from the fact that the precision of the method was not notably altered during this period it was concluded that by the measurement technique used such a possible effect did not interfere with the reliability of data obtained

As was anticipated from earlier observations (BERGSTROM & HENRIKSON 1970) the influence from gingival swelling on total attenuation has to be taken

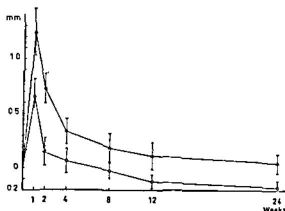


Fig 4 Changes in thickness with time in relation to preoperative values △ mean and SE for nine flap operations and ○ for nine gingivectomies

Compared to the initial changes characterized by bone loss the recovery phase is a rather slow process On an average for the total material the preoperative level of bone mineral mass is reestablished after about six months

Histologic observations on the reaction of the alveolar bone following periodontal surgery have been made both in animals (WILDERMAN et coll 1960, STAFFILENO et coll 1962, 1966, WILDERMAN 1963, CAFESSE et coll 1968) and in man (RAMFJORD & COSTICH 1963, 1968, PFEIFER 1963, 1965) The severity of the reaction is dependent upon the surgical technique and to which extent the bone has been exposed Experimental periodontal flap procedures in animals (WILDERMAN et coll 1960, WILDERMAN 1963) indicate that bone resorption as reflected by the osteoclastic activity was most extensive six to ten days after surgery, while histologic criteria of bone regeneration seem to suggest that this process would be completed within 2 to 3 months after surgery (WILDERMAN 1963, CAFESSE et coll 1968) In comparison with this histologic information—although not fully adequate, as the histologic findings are mostly based on animals with healthy periodontia—the present results would indicate that the processes of initial demineralization, and particularly that of the later remineralization, seem to be slower than and lag behind histologically evidenced stages of bone resorption and bone formation

The present results are in concordance with recent concepts of bone tissue dynamics (FROST 1964, URIST 1969, HAM & HARRIS 1971, RASMUSSEN & BORDIER 1973) After injury to the tissue, mesenchymal cells will be activated to form osteoclasts, producing resorption This phase lasts one or a few weeks At the end of this phase osteoblasts are produced (FROST 1964, RASMUSSEN & BORDIER 1973) Once the osteoblasts are formed, a new organic matrix is produced which subsequently becomes mineralized (PRITCHARD 1972) According to FROST (1964) mineralization begins one to two weeks after the matrix is formed and is completed after 3 to 4 months

Therefore, using a time scheme for alveolar bone healing in terms of mineral

mass analogous to that of WILDERMAN (1963), it seems appropriate to call the initial reaction, 0 to 30 days, the demineralization stage (corresponding to the preparatory, osteoclastic and osteoblastic stages of WILDERMAN) and consequently the period 30 to 180 days after the injury the remineralization stage (corresponding to the maturation stage)

The quantitative analysis of mineral mass changes of the alveolar bone reported was performed in its marginal interdental part The height of the remaining margin was reduced by, on an average, 15 per cent compared to normal, which is generally regarded to be about 90 per cent of the root length (Table 1)

These regions varied in mineral mass within rather wide limits (0 56 to 4 48 mg mm⁻²) Such interindividual variation may in part be qualitative but may also represent the orientation of the radiation beam within the subject on passage through the alveolar process, and particularly its relation to the alveolar margin

It has been reported (WILDERMAN et coll 1960, WILDERMAN 1963, WOOD et coll 1972, TAVTIGIAN 1970) that the margin is especially sensitive to resorptive changes after surgery In the present material no significant correlation was found between the presurgical amount of the alveolar bone and the severity of the initial reaction (expressed as loss of mineral mass) Further measurements, however, should be made to evaluate the magnitude of such changes for bone layers at different distance from the margin

On the other hand there was rather close conformity of the type of reaction between the two measurement positions within a certain region and at the given height level This indicates that possible erroneous influences due to a contribution to attenuation from dentine and cementum of adjacent teeth were limited, if at all present As the apparatus was constructed also for film exposure by the extremely collimated radiation, the tissue structure at the positions selected for recording could be examined Bone structure was demonstrated in the films of all areas

As was demonstrated by FORSBERG & HAGGLUND (1957) the trauma from periodontal surgery will result in an at least temporarily increased mobility of the teeth The increased tooth mobility may affect the reliability of the value of mineral mass obtained during the first postoperative period However, judging from the fact that the precision of the method was not notably altered during this period it was concluded that by the measurement technique used such a possible effect did not interfere with the reliability of data obtained

As was anticipated from earlier observations (BERGSTROM & HENRIKSON 1970) the influence from gingival swelling on total attenuation has to be taken into consideration when evaluating the changes pertaining to the mineral mass in vivo This is particularly true during the first four postoperative weeks and

when the procedure is a flap operation. As is reported elsewhere (BERGSTRÖM 1974) the oedema after surgery is more marked at a distance from than near the margin. The magnitude of such changes in the transverse thickness is thus dependent, among other things, also on the vertical height level of the measurement position.

To conclude, the present results reveal that periodontal surgery, whether gingivectomy or periosteal flap operation, brings about an average of 10 to 15 per cent loss of mineral mass in the interdental alveolar bone tissue. This is an insult to an already fragile bone tissue due to chronic periodontitis. In general, however, the tissue seems to repair within a six-month period. Whether this process of regeneration will continue in time to produce a more mineralized bone as a final result of the treatment demands further investigation.

SUMMARY

The influence on the alveolar bone mineral mass of two common techniques of periodontal surgery, mucoperiosteal flap operation and gingivectomy, was investigated in patients with periodontal disease. The bone reaction, similar with respect to type of treatment, was characterised by a loss of mineral mass during three to four weeks after surgery. The maximum loss was $0.3\ \text{mg}\cdot\text{mm}^{-2}$ on an average (10 to 15 per cent). A remineralization of the alveolar bone followed towards the presurgical level, which was reattained after four to six months.

ZUSAMMENFASSUNG

Es wurde der Einfluss von zwei gewöhnlichen Techniken der periodontalen Chirurgie, die mukoperiosteale Lappenoperation und die Gingivektomie, auf den alveolären knöchernen Mineralgehalt bei Patienten mit einer periodontalen Erkrankung untersucht. Die Knochenreaktion, die ähnlich im Hinblick auf den Typ der Behandlung war, zeichnete sich durch einen Verlust des Mineralgehalts während drei bis vier Wochen nach der Operation aus. Der maximale Verlust betrug im Durchschnitt $0.3\ \text{mg}\cdot\text{mm}^{-2}$ (10—15 %). Es erfolgte eine Remineralisation des alveolären Knochens bis zum präoperativen Gehalt, der nach vier bis sechs Monaten wieder erreicht war.

RÉSUMÉ

L'influence sur la masse minérale de l'os alvéolaire de deux techniques courantes de chirurgie periodontale, l'opération du volet mucopérioste et la gingivectomie, a été etudiée chez des malades atteints de maladie du periodonte. La réaction osseuse, semblable quelque soit le type de traitement, a ete caracterisée par une perte de la masse minérale pendant trois a quatre semaines apres l'intervention. Le maximum de perte a ete de $0.3\ \text{mg}\cdot\text{mm}^{-2}$ en moyenne (10 a 15 pour cent). La reminéralisation de l'os alvéolaire fait à l'opération et atteint le niveau preoperatoire au bout de quatre à six mois.

REFERENCES

BERGSTROM J An investigation of gingival topography in man by means of analytical stereophotogrammetry II Changes following periodontal surgery Acta odont scand 32 (1974), 221

– HENRIKSON C O Quantitative longitudinal study of alveolar bone tissue in man I Changes in alveolar bone tissue following periodontal surgery as recorded by an iodine 125 source J periodont Res 5(1970), 237

CAFFESSE R, RAMFJORD S and NASJLETI C Reverse bevel periodontal flaps in monkeys J Periodont 39 (1968), 219

FORSBERG A och HAGGLUND G Om parodontopati och tandrorlighet (In Swedish) Svensk tandlak T 50 (1957), 67

FRIEDMAN N and LEVINE L Mucogingival surgery current status J Periodont 35 (1964), 5

FROST H M The mathematical elements of lamellar bone remodelling Ch Thomas, Springfield, Illinois 1964

GLICKMAN I Clinical periodontology Saunders, Philadelphia, London, Toronto 1972

GOLDMAN H and COHEN W (eds) Periodontal Therapy, 4th ed Mosby, St Louis 1968

HAM A and HARRIS R Repair and transplantation of bone *In* The biochemistry and physiology of bone Vol III Edited by G H Bourne Academic Press New York and London 1971

HENRIKSON C O Iodine 125 as a radiation source for odontological roentgenology Acta radiol (1967) Suppl No 269

– and BERGSTROM J Quantitative long term determinations of the alveolar bone mineral mass in man by ^{125}I absorptiometry I Accuracy and precision of the method Acta radiol Ther Phys Biol 13 (1974), 377

and JULIN P Iodine apparatus for measuring changes in X ray transmission and the thickness of alveolar process J periodont Res 6 (1971), 152

OWNELL K A Quantitative roentgenologic studies on changes in mineral content of bone in vivo Acta radiol (1957) Suppl No 148

PFEIFER J The growth of gingival tissue over denuded bone J Periodont 34 (1963), 10

– The reaction of alveolar bone to flap procedures in man Periodontics 3(1965), 135

PRITCHARD J The osteoblast *In* The biochemistry and physiology of bone Vol I Edited by G H Bourne Academic Press, New York and London 1972

RAMFJO[illegible]

– – I [illegible]

RASMUS[illegible]

Mec[illegible]

STAFFILENO H LEVY S and GARGIULO A Histologic study of cellular mobilization and repair following a periosteal retention operation via split thickness mucogingival flap surgery J Periodont 37 (1966), 117

– WENTZ F and ORBAN B Histologic study of healing of split thickness flap surgery in dogs J Periodont 33 (1962) 56

TAVTIGIAN R The height of the facial radicular alveolar crest following apically positioned flap operations J Periodont 41 (1970) 412

URIST M R Mesenchymal cell reactions to inductive substrates for new bone formation *In* Repair and regeneration Edited by E Dunphy and W von Winkle McGraw Hill New York Toronto, Sydney and London 1969

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The influence on the alveolar bone mineral mass of two common techniques of periodon tal surgery, mucoperiosteal flap operation and gingivectomy, was investigated in patients with periodontal disease The bone reaction, similar with respect to type of treatment was characterised by a loss of *mineral mass* during *three to four weeks after surgery* The maximum loss was 0 3 mg mm on an average (10 to 15 per cent) A remineralization of the alveolar bone followed towards the presurgical level which was reattained after four to six months

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L influence sur *la* masse minerale de l os *alveolaire de deux techniques courantes* de chirurgie periodontale, l operation du volet mucoperioste et la gingivectomie a éte etudiee chez des malades atteints de maladie du periodonte La reaction osseuse semblable quelque *soit le type de traitement* a ete *caracterisee par une perte* de la masse minerale pendant trois à quatre semaines apres l intervention Le maximum de perte a ete de 0 3 mg mm en moyenne (10 à 15 pour cent) La remineralisation de l os alveolaire fait à l operation et atteint le niveau preoperatoire au bout de quatre à six mois

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RADIATION SENSITIZING EFFECT OF HEAT

K. OVERGAARD and J. OVERGAARD

In the efforts of finding means to improve the tumour-deleting effect of a certain amount of actinic energy (sensitization) a number of physical, biologic and pharmacologic factors have been analysed but not thoroughly the possible factor heat.

A considerable number of investigations—covering all steps of a scale from human tumours through experimental implanted tumours and various forms of cultured cells to special cellular organelles or enzymatic functions—have nearly unequivocally provided evidence suggesting that heat affects malignant tissues in a different and more deletory way than normal tissue

Although a large number of attempts to cure inoculated tumours in animals by heat have been successful, no real information has been gained as to the temperature and time relations which may determine such an effect, because continuous control of the intra tumoural temperature has either been omitted or has been technically imperfect

No thorough investigations of the histologic reaction of heat treated tumour tissue have been reported, and the way in which heat may affect such tissue is quite unclear In spite of these shortcomings reports compiled from the literature are highly suggestive as to some therapeutic possibilities of a heat treatment, and

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WILDERMAN M Repair after a periosteal retention procedure J Periodont 34 (1963), 487
— WENTZ F and ORBAN B Histogenesis of repair after mucogingival surgery J Periodont 31 (1960), 283
WOOD D, HOAG P, DONNENFELD W and ROSENFELD L Alveolar crest reduction following full and partial thickness flaps J Periodont 43 (1972), 141

FROM THE INSTITUTE OF CANCER RESEARCH, THE DANISH CANCER SOCIETY, RADIUMSTATIONEN, AND THE DEPARTMENT OF CELL BIOLOGY, THE INSTITUTE OF ANATOMY, UNIVERSITY OF AARHUS, DK-8000 AARHUS, DENMARK.

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in a previous report the question of a systematic analysis was considered (OVERGAARD & OVERGAARD 1972 a).

After the development of a tolerably reliable experimental technique, giving a possibility of continuous measurement and correction of the intra-tumoural temperature, a number of mice inoculated with an anaplastic mammary carcinoma were treated. In the temperature range of 41.5 to 43.5° C a reproducible number of cures was obtained. A relationship between the temperature used and the time of application required was demonstrated.

The clinical course was described, and a special histologic and histochemical reaction of the tumour tissue was demonstrated, suggestive of the mode of reaction. The reaction was selectively bound to tumour cells, and in successful cases all tumour cells were destroyed without any visible injury to the surrounding and interjacent normal cells.

These results, obtained exclusively by heat, may probably be elaborated for the use in human clinical practice—and, in fact, some cures of human tumours obtained by a similar treatment have been reported (CAVALIERE et coll. 1967).

However, the application of heat of sufficient intensity may be difficult and may cause discomfort. Under these circumstances some previous observations were revised and analysed, which suggested the existence of a synergistic effect of the combination of heat and roentgen irradiation (OVERGAARD 1935, OVERGAARD & OKKELS 1940, OVERGAARD & OVERGAARD 1972 b).

Method

If any synergism between heat and roentgen irradiation exists, it should be expected that combined heat—roentgen (cHR) treatment with doses of both components of a size expected separately to give no or only a few cures, may result in a definite increase in the curative effect. If that be the case, a subsequent investigation of the clinical course and of the microscopic appearances of the tumour may give suggestions as to the nature of this reaction.

An anaplastic mouse mammary carcinoma (HB) originating from the C3H strain of our Institute was implanted into the flank or inguen of C3H mice weighing 20—23 g. Untreated mice with a palpable 'take' all died with a large local tumour within 3 to 6 weeks. Metastases were few.

Tumours measuring about 5 mm × 5 mm × 6—8 mm (6 to 8 days after implantation) were used. Local heat was applied to the tumour by short-wave diathermy (27.12 Mc) using the technique previously described with continuous strict control of the intra-tumoural temperature (OVERGAARD & OVERGAARD 1972 a).

Table 1

Number of cures by local heat treatment. All cures without secondary injury

	42.5°/60	42°/120	41.5°/240	41°/480
Total surviving animals	40	18	21	5
Cures	9	4	8	2
Per cent	22	22	38	40

The term heat dose (HD) signifies the combination of tumour temperature and exposure time and is designed as a fraction, the numerator showing treatment temperature in centigrades, the denominator exposure time in minutes.

The tumour field was irradiated locally by a conventional roentgen therapy unit, the rest of the animal was shielded by 4 mm Pb. The size of the irradiated field was 1 to 2 cm^2 (factors: 250 kV, 15 mA, 36 cm skin-focus distance, 400 R inc/min). The dose is expressed in R inc.

All treatments were performed with the animals in Nembutal anaesthesia, 72 mg/kg/h being given intraperitoneally.

Whereas the roentgen technique is safe and gives a homogeneous affection of all parts of tumour, the heating technique is less perfect, as many non-controllable factors in the shape and site of the tumour may influence the heat application in some parts of the tumour and may disturb the results obtained.

To secure homogeneity of the material all the individual dose groups were compiled from single, or small numbers of, cases scattered over the total period of experimentation.

All tumours deviating from the usual mode of growth were excluded, so that the age and size of treated tumours were uniform. At least three animals were selected as controls in every inoculation batch (20 to 25 mice). The homogeneity of the relevant qualities of tumour was frequently controlled during the period of investigation.

Table 2

Effect of roentgen irradiation. All cures accompanied by heavy atrophic and fibrotic alterations

No. of surviving animals	Dose in R	Cures	Per cent
12	3 200	1	8.5
80	2 400	5	6.5
17	1 600	0	0
12	1 200	0	0

Table 3

Material treated with combined heat and roentgen irradiation Material arranged in head groups partly compiled of smaller series in different temperature levels with heat doses of presumably equivalence according to StHD Series with uniform dose components compiled without regard to variations in sequence and interval Concurrent controls of curative effect of treatment components mentioned Results recorded in fractions number of cures/number of surviving animals Cure rate in per cent valid for the head group

Heat dose StHD	Specificated HD	Roentgen irradiation 0 R	200 R		400 R
0	0	0/800			
1/8	40 5°/120	1/52			
	41°/60	0/21			
	41 5°/30	0/24			1/19
	42 5°/7 5	0/20			
1/4	41°/120	0/29			
	41 5°/60	1/15			
	42°/30	0/34			
	42 5°/15	1/35	3/44	(7 %)	
1/2	42°/60	1/33			
	42 5°/30	2/38	1/32	(3 %)	
1/1	42°/120	4/18			
	42 5°/60	14/66	3/20		
2	42 5°/120	1/17			

The experimental procedures killed a number of animals—either at once or within a few days All animals that died before 7 days were excluded as the curative effect at this period is impossible to assess

A total and lasting cure without any signs of tumour anywhere at least 6 months after treatment was considered the only objective criterion for success, all animals which did not fulfil these conditions were counted as failures Most of the latter animals died with evident tumour growth before 6 weeks Only very few died during the rest of the observation period without autopsic signs of tumour All cured animals were finally killed and subjected to autopsy, and microscopy of the tumour site performed No signs of tumour were revealed

Preliminary experiments

Initially, the curative heat dose (cHD) was determined in the range 41 to 42 5° C (Table 1) A higher HD appeared to increase the effect only slightly Downwards, the curative effect is not sharply defined, but one half of the cHD or less results in only very few or no cures (Table 3)

Table 3 *(cont)*

	Roentgen irradiation					
	800 R		1200 R		1600 R	
	0/31		0/70		0 81	
					11/48	
			9 68		5/34	
	18/217					
(5 %)	0/12	(8 %)		(13 %)	2/21	(17 5 %)
					6/27	
	9 60		36/153			
	4/26					
	10/43	(17 5 %)	3 8	(24 %)	7/27	(24 %)
			23/105			
	35/146	(24 %)	6/24	(22 %)	11/26	(42 %)
			9/13			
	6/19	(31 %)	1/5	(55 %)	17/26	(65 %)
					5/8	(62 %)

In a logarithmic system, the values of cHD observed form a straight line As all these combined values of temperature and exposure time presumably are equivalent in curative effect, and as equal fractions of the different cHD evidently provoke equal alterations in the tumour tissue (independent of the temperature range), it was found convenient (in spite of the limited material) to use this line as a working unit, the standard heat dose (1 StHD), in the combination of the physical influence and the biologic effect in this respect Possibly, it may be valid in comparing effects of HD given at different temperature levels in the actual investigation and useful in comparative investigations to other experimental tumour systems The validity of this assumption may be confirmed in further experiments

Microscopy of tumours treated with *subcurative doses* corroborated the previous findings destructive and mitotic alterations of a varying nature were present in all cases The number of *affected cells* and the severity of the affections accorded well with the dose given, independent of the actual temperature In all cases, a number of tumour cells were unaltered, and multicentric new-growth of tumour appeared within a few days

Table 4

cHR series treated with uniform total doses of components, but varied according to sequence of components and time of intervals H heat dose, R roentgen dose, + interval 0—4 h, (24) interval 24 h

H	R	H+R	H (24) R	R+H	R (24) H
41.5/30	800	6/72 (8.3 %)	5/63 (8 %)	5/47 (11 %)	2/35 (5.7 %)
41.5/60	800	3/19 (16 %)		3/20 (15 %)	3/21 (14 %)
41.5/60	1 200	3/14 (21 %)	28/118 (23.5 %)		5/21 (23 %)
42/60	1 200	5/22 (23 %)	10/40 (25 %)	4/22 (18 %)	4/21 (19 %)
42.5/30	800	10/52 (19.5 %)	7/23 (30 %)	7/27 (26 %)	11/44 (25 %)

The sensitivity of the tumour to roentgen irradiation was also estimated (Table 2) In most cases, some regression of the tumour and a transitory retardation of growth occurred after irradiation, followed by newgrowth of tumour Varying with the size of the dose applied the survival time could be prolonged 2 to 3 months or more Severe fibrotic and atrophic alterations were present in all cured animals No cures were obtained by doses of 1 600 R or less

Microscopically, all these doses produced characteristic alterations as commonly described At 1 600 R or less, a large number of mitotic irregularities were observed, accompanied by different degrading cell formations Extensive newgrowth of tumour cells occurred, usually within 1 to 2 weeks

Separately, all the mentioned heat and roentgen doses gave rise to distinct alterations in the tumour tissue, but by doses below the curative level only a negligible number of cures may be expected

Results

In the special investigation, a total of 2 881 tumour-bearing animals were used Of 1 467 animals treated with cHR 236 mice succumbed during treatment or within the next 7 days Of 1231 mice living at least 7 days, 241 survived for 180 days or more, presenting no signs of tumour at autopsy and local microscopy, while 982 animals which died with a definite tumour (nearly all within 6 weeks after the treatment) and 8 mice which died without a demonstrable tumour before 180 days were counted as failures The rough rate of cures was 16.5 per cent

Moreover, 614 mice were treated with only one of the two components in order to control the efficacy 586 died with tumours 800 untreated tumour-bearing controls all died with tumours

By successive local exposure of tumours to both components in stepwise varied doses in this range a considerable number of cures were obtained (Table 3)

Here the heat doses are arranged in large groups consisting of subgroups in different temperature ranges of hypothetical equivalence (according to the size of the fraction at the StHD) The validity of this arrangement may be queried The physical roentgen doses, on the other hand, may be estimated as biologically equivalent in the respective columns

In most cases, the heat application was followed by roentgen irradiation for half an hour to four hours, but in some cases the sequence of the components was reversed Furthermore, in some experiments (of both variations) the interval between the exposures was prolonged to 24 h No significant difference in the curative effect was revealed by these variations (Table 4)

After the treatment, no local or general reaction was observed During the next few days, the tumour became firmer in consistency The size was mostly unchanged or decreased slightly This state may remain almost unaltered for about 2 to 3 weeks, then the tumour hardened, became smaller and, finally, *totally disappeared The skin may be unaltered during this process, but in many* cases (about one third of the animals) some infiltration of the skin formed a crust over the tumour or possibly included some of its circumference The diameter varied from 2 mm to about 1 5 cm This crust usually persisted for a couple of months, then gradually loosened from the border leaving a plain epithelial scar, finally covered with discoloured hair

In animals which were not cured the initial reaction was quite identical, but some newgrowth may be palpated at the border or base of the tumour, in most cases within 1 to 2 weeks The growth of this was like that of a normal tumour, and the animal always succumbed in 3 to 6 weeks

Extensive microscopy of tumour tissue after cHR treatment revealed some special alterations clearly deviating from the reactions known after isolated heat or roentgen applications In all, the more hypoxic and denser central parts of the tumour, total necrosis developed within a few hours On the other hand, a narrow peripheral brim of active A cells revealed a greatly protracted necrobiotic decay The most characteristic feature here was—in successful cases—the total absence of all mitotic activity in tumour cells Histochemical examinations indicated the participation of an early and vigorous lysosomal activity in the tumour cells (A detailed report on the microscopic findings will be published in a subsequent paper)

Discussion

The present material clearly demonstrates the possibility of *curing an implanted malignant tumour* by successive application of highly subcurative doses of two different tumour-destructive agents, some synergism between heat and irradiation exists

In addition to the demonstration of this possibility the clinical material gives only sparse information concerning the nature of this reaction.

While curative effect of the heat dose is demonstrated as low as 1/8 StHD (and in fact exists at even lower levels) the effect of doses in the size 200 to 400 R is unsafe. Transient clinical reaction of tumour was normally observed and microscopic alterations were constant but cures were few.

As a whole, the complex of variations of doses in Table 2 shows a tendency to enhancement of the curative effect by an increase of each factor separately or of the two factors together. However the significance of the quantitative relations is not sufficiently clarified. In several cases subgroups of presumably equivalent dose combinations in different temperature ranges give very concordant results, in other cases a wide discrepancy exists. As most of this dissimilarity may be levelled out by using the totals of the main groups as standards of reference a larger material may settle the disturbing influence of the aforementioned non-controllable (heat) factor(s).

It must be emphasized that in all recurrent cases almost all tumour tissue was primarily destroyed and newgrowth started in some peripheral part of the tumour. Presumably some deficiency in the heat application may here be the cause. Further investigations are in progress.

SELAWRY et coll. (1958) have surveyed previous literature on this subject satisfactorily. Reports of about 500 cases of human malignant tumours treated with some form of combined heat—irradiation application are published. Most reports are from the period 1910—35, the informative value is usually low. Attention may be drawn to two groups. DOUB (1935), WARREN (1935) and SHOULDERS et coll. (1942) reported well established palliative results in about 60 far advanced cases of human malignant tumours by a general hyperthermia at about 42° C and moderate roentgen doses. No cures were claimed. Similar results obtained by intravesical heating up to 43° C were reported by COCKETT et coll. (1967) in advanced cases of bladder tumours.

WOEBER & STEIN (1963) reduced the curative roentgen dose in skin carcinomas by 33 to 45 per cent by simultaneous addition of ultrasound—heating.

By the use of high frequency currents (in different forms) a high effect, even cure, have been claimed, no long term investigation confirm this, and more realistic reports (KORB 1948, *BIRKNER & WACHSMANN 1949*) *give evidence* of some palliative effect but mostly draw attention to the technical difficulties in adequate application and measurement of heat. Presumably these obstacles have *not been overcome*. Mostly a *low effect* was obtained by use of hyperthermic immersion (DALICHO 1957, KIRSCH & SCHMIDT 1967). In addition to some in vitro experiments suggesting a heat—roentgen synergism a moderate number of *in vivo* experiments have confirmed the aforementioned observation

During recent years, CRILE (1963), ARDENNE (1969), MUCKLE & DICKSON (1973), ROBINSON et coll (1972) have reported similar results, using an immersion technique, only the two last mentioned controlled the intra-tumoural temperatures Two experimental reports (CLARKE et coll 1970, TAYLOR 1936) could not confirm such relations

There seems to be no possibility of comparing our treatment doses and results with previous observations In most of these, the HD is uncertain and nearly all investigations were performed in not absolutely isologous systems Although some parallelism in action may exist, a number of extraneous factors may influence the tumour reaction The material studied by ROBINSON et coll is an exception, but their analysis is rather summary, giving no possibility of estimating the number of probable cures

In the total cHR reaction, many single elements may be traced back to alterations already known in the heat or irradiation reactions Nevertheless, in addition to the differences in the curative effect and the clinical course, the microscopic appearances indicate a mode of action which is different from that of each of the two components

Under such circumstances it is suggested that the combined heat—roentgen effect may depend on a synergism of the actions of the two individual components

To estimate the common validity of this reaction similar experiments have been performed in a smaller scale on 10 other tumour systems In general the main features in the reaction was confirmed, but special conditions in the different tumours might influence the curative possibility and microscopic details

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SUMMARY

The radiation sensitizing effect of heat is analysed In an isologous mouse tumour system a successive local exposition to heat and roentgen irradiation with doses of both components of a size expected separately to give no or a few cures only an evident rise in the curative results was obtained The presence of a synergism of the factors applied is suggested The effect obtained depends on the dose of both factors It is independent of the sequence of the application of the two components and is not reduced by an interval of 24 h between the exposures

ZUSAMMENFASSUNG

Der strahlensensibilisierende Effekt von Warme wird analysiert An einem isologen Maus Tumor System ergab eine aufeinander folgende lokale Exposition mit Warme und Ront-

genbestrahlung mit Dosen beider Komponenten, bei denen keine oder nur wenige Heilungen zu erwarten waren, einen deutlichen Anstieg der kurativen Ergebnisse. Das Vorkommen eines Synergismus der verwendeten Faktoren wird vermutet. Der erhaltene Effekt hängt von der Dosis der beiden Faktoren ab. Er ist unabhängig von der Sequenz der Applikation der beiden Komponenten und wird nicht durch ein Intervall von 24 Stunden zwischen den Expositionen vermindert.

RÉSUMÉ

Les auteurs ont étudié l'effet radiosensibilisant de la chaleur. L'exposition locale d'un système isologue souris-tumeur à des doses de chaleur et d'irradiation roentgen qui séparément ne donneraient aucune guérison ou seulement quelques guérisons donnent lieu à une augmentation évidente des résultats curatifs. Les auteurs pensent qu'il y a une synergie de ces deux facteurs. L'effet obtenu dépend de la dose de ces deux facteurs. Il est indépendant de l'ordre dans lequel sont appliqués ces deux agents et n'est pas réduit par un intervalle de 24 heures entre les expositions.

REFERENCES

ARDENNE M. v., CHAPLAIN R. A. und REITNAUER P. G.: In-vivo-Versuche zur Krebs-Mehrschritt-Therapie mit der Attackenkombination optimierte Tumorübersäuerung + Hyperthermie + schwache Röntgenbestrahlung. Dtsch. Gesundh.-Wes. 24 (1969), 924.

BIRKNER R. und WACHSMANN F.: Über die Kombination von Röntgenstrahlen und Kurzwellen. Strahlentherapie 79 (1949), 93.

CAVALIERE R., CIOCATTO E. C., GIOVANELLI B. C., HEIDELBERGER C., JOHNSON R. O., MARCOTTINI M., MONDOVI B., MORICCA G. and ROSSI-FANELLI A.: Selective heat sensitivity of cancer cells. Cancer (Philad.) 20 (1967), 1351.

CLARKE P. R., HILL C. R. and ADAMS K.: Synergism between ultrasound and X rays in tumour therapy. Brit. J. Radiol. 43 (1970), 97.

COCKETT A. T. K., KAZIM M., NAKAMURA R., FINGERHUT A. and STEIN J. J.: Enhancement of regional bladder megavoltage irradiation in bladder cancer using local bladder hyperthermia. J. Urol. (Baltimore) 97 (1967), 1034.

CRILE G. JR.: The effects of heat and radiation on cancers implanted on the feet of mice. Cancer Res. 23 (1963), 372.

DALICHO W. A.: Klinische Erfahrungen mit lokalen und allgemeinen Überwärmungen maligner Tumoren in Kombination mit der Strahlentherapie. *In:* Körpereigene Abwehr und bösartige Geschwulste, p. 109. Herausgegeben von H. Lampert and O. Selawry. Karl F. Haug Verlag, Ulm-Donau 1957.

DOUB H. P.: Osteogenic sarcoma of clavicle treated with radiation and fever therapy. Radiology 25 (1935), 355.

GIOVANELLI B. C., MORGAN A. C., STEHLIN J. S. and WILLIAMS L. J.: Selective lethal effect of supranormal temperatures on mouse sarcoma cells. Cancer Res. 33 (1973), 2568.

KIRSCH R. und SCHMIDT D.: Erste experimentelle und klinische Erfahrungen mit der Ganzkörper-Extrem-Hyperthermie. *In:* Aktuelle Probleme auf dem Gebiet der Cancerologie, p. 53. Herausgegeben von M. v. Ardenne. Springer, Berlin und Heidelberg 1967.

KORB H.: Über eine Kombination der Röntgenbestrahlung mit der Kurzwellenbehandlung. Strahlentherapie 77 (1948), 301.

MUCKLE D S and DICKSON J A Hyperthermia (42° C) as an adjuvant to radiotherapy and chemotherapy in the treatment of the allogenic *VX-2* carcinoma in the rabbit Brit J Cancer 27 (1973), 307

OVERGAARD K Experimentelles über kombinierte Wärme-Röntgentherapie bösartiger Tumoren Acta radiol 16 (1935), 461

— und OKKELS H Über den Einfluss der Wärmebehandlung auf Woods Sarkom Strahlentherapie 68 (1940), 587

— and OVERGAARD J (a) Investigations on the possibility of a thermic tumour therapy I Short wave treatment of a transplanted isologous mouse mammary carcinoma Europ J Cancer 8 (1972), 65

— — (b) Investigations on the possibility of a thermic tumour therapy II Action of combined heat roentgen treatment on a transplanted mouse mammary carcinoma Europ J Cancer 8 (1972), 573

— — *Histologic reaction in a mouse mammary carcinoma following exposure to combined* heat roentgen irradiation To be published in Acta radiol Ther Phys Biol

ROBINSON J E, WIZENBERG M J, McCREADY W and SCHELTEMA J The third international conference on medical physics including medical engineering Chalmers University of Technology, Gothenburg Sweden, 1972 Radiat Biol 1 (1972), 39

SELAWRY O S, CARLSON J C and MOORE G E Tumor response to ionising rays at elevated temperatures Amer J Roentgenol 80 (1958), 833

SHOULDERS H S, TURNER E L, SCOTT L D and GRANT W H Effect of combined fever and X ray therapy on far advanced malignant growths Radiology 39 (1942) 184

STROM R SANTORE A CRIFO C BOZZI A MONDOVI B and FANELLI A R The biochemical mechanism of selective heat sensitivity of cancer cells Europ J Cancer 9 (1973), 103

TAYLOR H J The effect of ultra high frequency currents (ultra short waves) combined with non lethal doses of radium radiations upon experimental rat tumours Brit J Radiol 9 (1936) 467

WARREN S L Preliminary study of effect of artificial fever upon hopeless tumor cases Amer J Roentgenol 33 (1935) 75

WOEBER K und STEIN G Ergebnisse bei kombinierter Röntgen und Ultraschallbehandlung bösartiger Hauttumoren Strahlentherapie 122 (1963), 285

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IRRADIATION OF SMALL STRUCTURES THROUGH THE INTACT SKULL

BORJE LARSSON, KURT LIDÉN and BERT SARBY

Narrow beams of penetrating ionizing radiation may be used to transfer radiation energy to affect small, well circumscribed tissue volumes in the depth of the brain for functional neurosurgery and for treatment of small benign tumours This kind of 'neurosurgical' operations through the intact skull is considered to give essential advantages over existing so-called 'open' *methods* (LEKSELL 1951, 1966) The physical conditions for the use of ionizing radiation for these purposes are today relatively wellknown, mainly through the work with the cyclotrons at Berkeley and at Uppsala (LARSSON 1962, LAWRENCE et coll 1962, TOBIAS et coll 1964, LARSSON & SARBY 1975) but, unfortunately, there exists no suitable construction that permits routine use *in the hospital* The technical and physical aspects of this problem have therefore been considered and a ^{60}Co apparatus proposed The work was done in close collaboration with the neurosurgeon (LEKSELL 1971)

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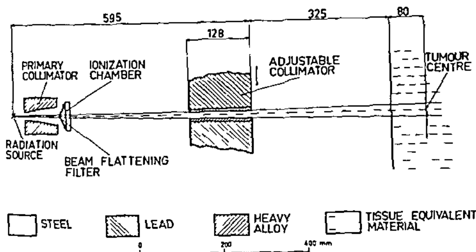


Fig 1 Experimental lay-out for determining with photographic dosimetry in a tissue equivalent phantom the distribution of absorbed dose at irradiations with 6 MV roentgen beams shaped to define geometrical cross sections of 10 mm × 10 mm and 30 mm × 30 mm

The term radiation surgery is here taken to represent various procedures employing localized irradiation for the destruction of small, mostly deep lying, regions of tissue, healthy or neoplastic, for therapeutic purposes Such techniques should be considered supplementary to various physical methods of open surgery employing local application of heat, cold, radiation from interstitial sources or ultrasound A first attempt to use external irradiation for functional surgery was made by LEKSELL et coll (1955) who reported on the use of conventional roentgen radiation and a stereotaxic instrument for tre

precise administration of necrotizing doses of radiation to circumscribed tissue volumes without untoward injury to surrounding structures LIDÉN (1957) thus presented a preliminary analysis of the physical possibilities and recommended the use of 10 to 20 MV roentgen radiation or high energy ion beams

The following requirements on the technique should be met

1) The apparatus should be so constructed that irradiation could be performed with sufficiently good precision in the administration of the desired dose to the localized target volume in the brain As a basis for establishing the physical requirements of the technique the conditions for similar operations

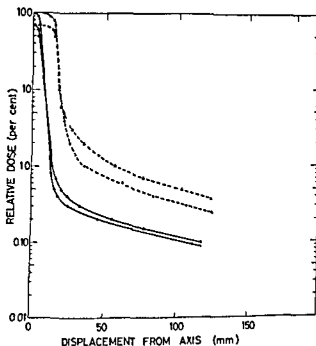


Fig. 2. Distribution of absorbed dose in a transverse plane at phantom depths of 1.5 cm (●—●—●, ○—○—○) and 8 cm (△—△, ▲—▲) for 10 mm and 30 mm wide 6 MV roentgen beams, respectively. The curves are normalized relative to the dose on the beam axis at a depth of 1.5 cm. The dose gradient in the penumbra regions of the beams is similar to that obtained for 2.5 mm proton and gamma beams (Fig. 1).

with the 185 MeV proton synchrocyclotron at Uppsala were accepted (Larsson et coll. 1963).

2) Clinical demands for acceptable treatment times and free space for the patient around the isocentre should be fulfilled.

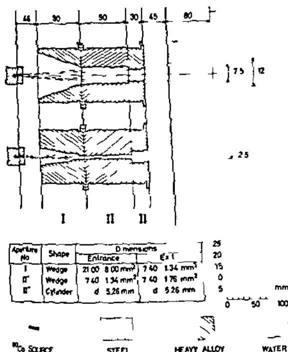
3) The radiation energy absorbed in the whole brain, that is the 'integral dose', should not be greater than that usually accepted as a tolerable single integral dose in radiation therapy of malignant brain tumours.

4) Current recommendations for radiation protection of the body of the patient and for the personnel should be fulfilled (ICRP 1970). This also includes arrangements needed to guarantee proper function of the apparatus at start and stop as well as continuous supervision of the patient.

To these criteria should be added a great many practical aspects on the construction of the apparatus, particularly in regard to the desired adaptation of available stereotaxic localization techniques (Leksell 1971).

Methods

Dose distribution determinations under simulated conditions were performed in narrow beams of 185 MeV protons, ^{60}Co gamma rays and 6 MV roentgen radiation (Figs. 1, 3). The centre of the target volume was assumed to lie at a depth of 8 cm in a tissue-equivalent phantom, the mean radius of a normal



Aperture No	Shape	Dimensions	
		Entrance	Exit
I	Wedge	21 00 8 00 mm²	7 40 1 34 mm²
II′	Wedge	7 40 1 34 mm²	7 40 1 76 mm²
II″	Cylinder	d 5 26 mm	d 5 26 mm

Fig 3 Experimental lay-out for determining with photographic dosimetry in a tissue-equivalent phantom the distribution of absorbed dose at irradiation with ^{60}Co beam shaped to define a geometrical cross section of 2 5 mm × 7 5 mm

skull The methods used were described in detail by LARSSON & SARBY (1975) and SARBY 1974

The geometrical demands on the single ^{60}Co gamma beam channel was discussed by SARBY (1974) In order to optimize the collimation of a narrow gamma beam a collimating arrangement was constructed in 17 2 g/cm³ heavy alloy (Fig 3) It consisted of one primary collimator (I) and two beam defining collimators (II and II) and it was mounted in an irradiation geometry similar to that of the discussed treatment apparatus

The roentgen beams were produced with a 6 MeV linear accelerator for radiation therapy (Varian V 7705 Clinac AUSTIN 1964) The irradiation geometry appears in Fig 1 The accelerator produced radiation with a mean dose rate of about 200 rad/min in pulses with a duration of 1 μs and a separation of 5 ms The diameter of the focus was approximately 3 mm

Calculation of superposed dose distributions as a result of irradiation with multiple beams was made with computer programs described by DAHLIN (1970) and DAHLIN & SARBY (1975) (Fig 7) The spatial distribution of the beams was defined by means of a coordinate system (Fig 6)

Measurements at the ^{60}Co apparatus The dose rate at the beam focus of the ^{60}Co unit was determined from measurements at the centre of a spherical plexiglass phantom (diameter 16 cm) with LiF plates (1 mm × 4 mm × 4 mm) The dosemeters were calibrated in a water phantom irradiated with a broad ^{60}Co gamma beam The doses were calculated according to a formula derived by BURLIN (1966) with an overall uncertainty of ± 5 per cent

Secondary radiation doses to various structures in the head and to different organs in the body were measured with LiF teflon rods (diameter 1 mm, height 4 mm) in a tissue-equivalent whole body phantom and on patients irradiated by the ^{60}Co unit The dosemeters were calibrated in a broad ^{60}Co beam and their energy dependence for the degraded photon energy in different parts of the body was not of significance for the results

Results and Discussion

Radiation

The radiation should have sufficient penetrability and little scattering in the passage through the tissues of the head to selectively destroy tissue volumes of diameters about 2 to 12 mm at depths of 6 to 10 cm The demands on the distribution of absorbed dose in the brain can be specified from the experiences of earlier attempts to produce useful radiation lesions in experimental animals or patients (LARSSON et coll 1963) and from the irradiation of brain tumours (LINDGREN 1963) (1) The absorbed dose in the target volume should be about 20 krad in one single irradiation, (2) The distribution of absorbed dose should have a large gradient in the border zone of the desired lesion, (3) Immediately outside the planned lesion the absorbed dose must be as low as possible and not exceed 5 krad, (4) The average dose in the brain should be as low as the conditions permit and should not be considerably higher than approximately 100 rad

The technical situation today in regard to radiation sources and radiation handling is of primary concern Omitting, for practical and economical reasons, the previously used light ions, i e high-energy protons, deuterons or alpha particles, there are four kinds of more easily available ionizing radiation the penetration of which should permit irradiation of target volumes near the centre of the head high-energy electrons, supervoltage radiation, gamma radiation from nuclides and fast neutrons All these radiations are, in principle, useful for the purpose However, the choice between them requests detailed consideration of their attenuation, absorption and scattering properties with the view towards the possibility of obtaining well-defined narrow beams during the passage through the tissues

Table 1

Possible gamma emitting nuclides for cerebral radiation surgery

Nuclide	Half life $T_{\frac{1}{2}}$ (day)	Photon energy* (abundance per disintegration) (MeV)	Mean energy E_a (MeV)	Photons per disintegration
^{46}Sc	84	0.89 (1.00) 1.12 (1.00)	1.00	2.0
^{60}Co	1 920	1.17 (1.00) 1.33 (1.00)	1.25	2.0
^{160}Tb	73	1.27 (0.07) 1.18 (0.15) 0.97 (0.31) 0.88 (0.31) 0.30 (0.30)	0.81	1.14
^{182}Ta	115	0.22 (0.08) 1.12 (0.34) 1.16 (0.03) 1.19 (0.16) 1.22 (0.45)	1.11	1.06
^{192}Ir	74	0.885 (0.005) 0.612 (0.06) 0.604 (0.09) 0.589 (0.04) 0.468 (0.49) 0.317 (0.81) 0.308 (0.30) 0.296 (0.29)	0.37	2.1

* Only important components are given

High energy electrons are similar to the protons previously used to some success for radiation surgery since they are uni-charged atomic particles travelling with a velocity approaching that of light. On their way through the tissues they interact almost continuously with the molecular electronic structure and thus energy is transferred to the tissue along near rectilinear tracks. Only the high-energy part of the electron track is of interest. The considerable spread of the beam through successive scattering of the particles make impossible the use of the last, say, 10 cm of the range for selective irradiation of small tissue volumes. Demanding that the radiation has to pass intervening tissues of not less than 8 cm thickness this condition means that we have to choose a range of at least approximately 20 cm which corresponds to a kinetic energy of 40 MeV. At this energy the electron has a mass 80 times larger than the mass at rest, a fact that explains why such particles can move nearly rectilinearly through tissue. Nevertheless, they are much more influenced by elastic multiple scattering against atomic nuclei than 185 MeV protons, which are, under the conditions considered, another factor of 25 heavier. The importance of these differences in regard to scattering is considerable. Besides of reducing the gradient of the dose distribution at the geometrical edges of the beam, the phenomenon

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The technical situation today in regard to radiation sources and radiation handling is of primary concern Omitting, for practical and economical reasons, the previously used light ions, i e high-energy protons, deuterons or alpha particles, there are four kinds of more easily available ionizing radiation the penetration of which should permit irradiation of target volumes near the centre of the head high-energy electrons, supervoltage radiation, gamma radiation from nuclides and fast neutrons All these radiations are, in principle, useful for the purpose However, the choice between them requests detailed consideration of their attenuation, absorption and scattering properties with the view towards the possibility of obtaining well-defined narrow beams during the passage through the tissues

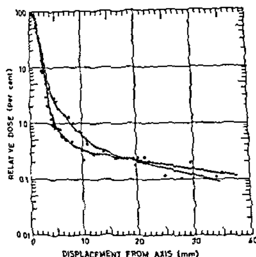


Fig 4 Distribution of absorbed dose in a transverse plane at a depth of 8 cm in tissue irradiated by a 2 5 mm wide ^{60}Co beam (●) under the conditions shown in Fig 3 For comparison the corresponding distribution for a 185 MeV proton beam (○) is given

Indeed, the experimental results revealed the usefulness of ^{60}Co gamma radiation (Figs 4, 5 and SARBY 1974) Approximately, the conclusions arrived at should be valid also for roentgen radiation from comparable electron accelerators This is evident from the measurements on 10 mm and 30 mm wide roentgen beams (Fig 2) The results (Fig 2) showed almost the same dose gradients in the penumbra region of the beams as those obtained for narrow 185 MeV proton beams and ^{60}Co gamma ray beams (Fig 4) The dose levels outside this region were also almost the same At higher energies of the roentgen radiation the diffusion of long range secondary electrons may seriously widen the penumbra region of the beams At higher energies the higher cost further becomes a significant disadvantage for this type of apparatus

The choice between the two alternatives i e roentgen or gamma radiation, should be based on technical, clinical and economical rather than physical considerations If radiation surgery will reach a position as a standard procedure, improved electron accelerators for roentgen production, adapted for the purpose, would seem a most attractive alternative

Nuclides Gamma emitting nuclides have some advantages in the present context Once installed, they are easy to handle and, most important, they permit unique reproducibility of the physical conditions during treatment The proposed apparatus for cerebral radiation surgery with a great number of individually radiating gamma sources was based on the data for the gamma-emitting nuclides presented in Table 1 With the availability of high flux density nuclear reactors giving 10^{13} to 10^{14} neutrons/cm^2 s, it has been possible

contributes to the strongly falling central depth dose distribution in an electron beam of otherwise suitable range. The shape of this curve is not known for relevant beam diameters but the depth dose curve of electrons (BRAHME 1971) would probably be significantly inferior to the corresponding curves for 185 MeV protons (LARSSON & SARBY) or ^{60}Co gamma radiation (SARBY). The fact that electrons in the energy range 30 to 40 MeV can be produced with commercially available small accelerators at reasonable cost means that they may become of practical interest for radiation surgery. However, electrons seem to offer no advantages over, for example, gamma radiation from ^{60}Co or supervoltage roentgen radiation. The latter type of radiation can also be produced by electron accelerators, at lower energy and cost.

Supervoltage roentgen radiation. At sufficiently high energy, roentgen radiation can be used for precise irradiation of tissues at the depth. Characteristic for such radiation is that the photons are distributed over a continuous energy spectrum in which the single photon can have an energy varying from that of the accelerated electrons down to a relatively low threshold. The shape of the spectrum is also dependent on target material and filtration (BRYNJOLFSSON & MARTIN 1971, JESSEN 1973). In the actual energy range, the mean energy of the photons is about one third of the electron energy. These circumstances mean, for example, that ^{60}Co gamma radiation, with an average energy of 1.25 MeV, is approximatively equivalent to roentgen radiation produced at an acceleration potential of 4 MV. Correspondingly, gamma radiation from ^{192}Ir, at an average energy of 0.37 MeV, may be said to correspond to 1 MV roentgen radiation.

From the principal point of view, therefore, it is not necessary to differentiate between roentgen and gamma radiation. Difficulties are more of a practical nature when the choice is to be made between roentgen radiation from electron accelerators and gamma radiation from nuclides. On one side the roentgen radiation from commercial linear accelerators for radiation therapy in the range 4 to 15 MeV permits high dose rates and well-collimated beams and they also give better flexibility because arbitrary directions of the beam relative to the head of the patient can be chosen in comparison with a ^{60}Co equipment that necessarily has to employ a multiple beam arrangement (vide infra). However, presently employed systems of acceleration and beam transport do not permit the desirable precision in the position and size of the focus and without further improvement they are not suitable for the purpose discussed. Further, the mechanical precision has to be improved to permit desired alignment of beam axis with the isocentre. Today the position of the isocentre of commercial apparatus is normally located within a sphere of 1 mm radius

Table 4

Activity per cm³ for the various nuclides at optimum conditions for activation and decay

Nuclide	Period of activation and decay $\tau = t$ (days ca.)	Activity per cm³, a_v according to equation 2 (Ci cm⁻³)			
		$\eta\,\varphi = 1$*	$\eta\,\varphi = 0.5$*	$\eta\,\varphi = 0.2$*	$\eta\,\varphi = 0.1$*
^{46}Sc	170	450	230	90	45
^{60}Co	1 000	1 800	900	360	180
^{160}Tb	150	730	370	150	73
^{182}Ta	230	580	290	120	58
^{192}Ir	150	14 000	7 000	2 800	1 400

* in 10^{14} neutrons per cm² and s

Table 5

The length of the sources and the total activity of the apparatus, calculated for 180 sources, cross section 0.008 cm², $f_b\,f_a = 0.2$ after activation with 0.5×10^{14} neutrons per cm² and s

Nuclide	Linear attenuation coefficient for the source material* (cm⁻¹)	Length of the sources L (cm)	Total volume of the sources (cm³)	Total activity in the apparatus	
				Nominal activity A_t (Ci)	Equivalent activity A_e (Ci)
^{46}Sc	0.173	5.3	7.2	1 700	2 200
^{60}Co	0.47	2.0	2.9	2 500	3 300
^{160}Tb	0.59	1.6	2.3	850	630
^{182}Ta	0.99	0.93	1.3	380	260
^{192}Ir	5.1	0.17	0.25	1 800	2 400

* Corresponding to the mean energy E_a of the photons

All the nuclides considered in Table 1 are produced by neutron capture reactions, the activity per cm³, a_v, produced (in Ci/cm³) after τ months of irradiation and t months of cooling being

$$a_v = 1630\,\frac{\varrho\, h\, \sigma\, \varphi\, s\, \eta}{A-1}\left(1-e^{-0.693\,\frac{\tau}{T_{\frac{1}{2}}}}\right)e^{-0.693\,\frac{t}{T_{\frac{1}{2}}}} \qquad (1)$$

where ϱ is the density (in g cm⁻³), h the concentration in the target (weight

Table 2

Data used for calculation of the activity per cm³ for the various nuclides

Mother nuclide (atomic mass A 1)	Weight fraction in the natural abundance of the element h	Density ρ (g cm⁻³)	Cross section for production of the daughter nuclides in Table 1 σ (barn)	Maximum activity per cm³ of the daughter nuclide a(max) (Ci cm⁻³)
^{45}Sc	1.00	2.9	23	2 400
^{59}Co	1.00	8.9	37	9 100
^{159}Tb	1.00	8.2	46	3 900
^{181}Ta	1.00	16.6	21	3 100
^{191}Ir	0.385	22.4	1 000	74 000

Table 3

Product of the build up factor and decay factor (f_b f_d) for various periods of activation (τ) and cooling (t) expressed in half lives ($T_{\frac{1}{2}}$)

$\frac{\tau}{T_{\frac{1}{2}}}$ \ $\frac{t}{T_{\frac{1}{2}}}$	0	0.25	0.50	1.0	2.0	3.0
0.25	0.159	0.134	0.112	0.080	0.040	0.020
0.5	0.293	0.246	0.207	0.147	0.073	0.037
1.0	0.500	0.421	0.353	0.250	0.125	0.063
2.0	0.750	0.631	0.530	0.373	0.188	0.094

to produce sources of various gamma-emitting nuclides of high specific activity, suitable for radiation therapy. It soon became clear that ^{60}Co was the only nuclide that today completely fulfils the demands. Its relatively long half-life, 5.3 years, suitable gamma energies, and relatively high production cross section are all convenient. Other possible nuclides considered have less suitable half-lives and cannot be easily produced in required amounts (Tables 1 to 6). The mechanical and chemical properties of the target material must also be taken into account.

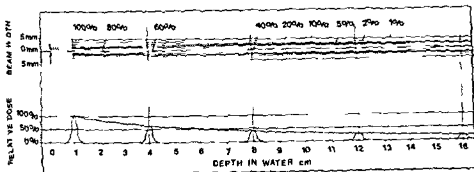


Fig 5 Isodose curves for a 2 5 mm wide ^{60}Co beam measured in a coaxial plane (the upper part of Fig 3) and normalized to 100 % at a depth of 1 cm

To arrive at an estimate of practically achievable dose rates we further assume the multiple beam device to contain 180 cylindrical sources (length L cm, cross section 0 008 cm^2) and that irradiation takes place under the condition $f_b\ f_d = 0\ 2$ and $\eta\ \varphi = 0\ 5 \times 10^{14}$ cm^{-2} s^{-1} The length of the sources (Table 5) was considered to be optimal when the utilization factor due to the influence of self absorption in the source was 65 per cent (SARBY 1974) Under these conditions the total activity A_t in the apparatus will be, for ^{60}Co, 2500 Ci (for comparison with other nuclides see Table 5) It is convenient to calculate the activity A_e of an equivalent point source, producing one gamma photon per disintegration With the source centre distance, 38 cm of the suggested apparatus (Figs 3, 8), and representing the head by a concentric tissue equivalent sphere of 8 cm radius, the dose rate D (rad h^{-1}) at the centre assuming narrow beam attenuation will be (SARBY 1974)

$$D = 117\ A_e\ E_a\ \mu_{en}\ e^{-\mu 8} \qquad (3)$$

where E_a (MeV) is the mean gamma energy per disintegration (Table 1) and μ_{en} and μ, respectively, the linear energy absorption coefficient and linear attenuation coefficient for water in cm^{-1} (Table 6) The result for ^{60}Co, 8700 rad h^{-1} is given in Table 6 together with data of the other nuclides considered

Cobalt 60 is thus presently to be considered the obvious choice although some of the other nuclides are potentially interesting Particularly ^{192}Ir with, theoretically, very high maximum activity per cm^3 and convenient mechanical and chemical properties is recommended for further testing especially when higher neutron flux densities will be available The disadvantageous short half life may be compensated for by designing convenient source exchange

Table 6

Dose rates at a depth of 8 cm in tissue at beam focus of an apparatus of the same geometry as the suggested ^{60}Co unit, calculated for sources according to Table 5

Nuclide	Equivalent activity A_e (Ci)	Mean energy of the photons F_γ (MeV)	Linear attenuation coefficient for tissue μ (cm^{-1})	Linear energy absorption coefficient for tissue μ_{en} (cm^{-1})	Dose rate D (rad h^{-1})
^{46}Sc	2 200	1.00	0.071	0.031	4 400
^{60}*Co*	*3 300*	*1.25*	*0.063*	*0.030*	*8 700*
^{160}Tb	630	0.81	0.078	0.032	1 000
^{182}Ta	260	1.11	0.067	0.030	590
^{192}Ir	2 400	0.37	0.11	0.033	1 400

fraction) and σ the cross section of the mother nuclide of atomic mass A−1 for neutron capture (in 10^{-24} cm^2) (LEDERER et coll 1967). The factor s (always close to 1) corrects for the decrease in concentration due to consumption of the mother nuclide in the process. Similarly, the factor η reflects the decreased efficiency of activation due to the fact that the efficient flux density in the target may be very different from the nominal flux density φ (in 10^{14} neutrons/cm^2 s) due to attenuation in target and capsule materials. Table 2 gives, besides the physical characteristics of the target material, the maximum activity per cm^3, a_{max}, which would be achieved at equilibrium after irradiation of 10^{14} neutrons/cm^2 s provided s = 1 and η = 1. For convenience, the build-up factor $f_b = \left(1-e^{-0.693\frac{\tau}{T_{1/2}}}\right)$ and the decay factor $f_d = e^{-0.693\frac{t}{T_{1/2}}}$ are given separately in Table 3. The activity per cm^3 achievable during varying conditions of activation can now be calculated from the formula

$$a_v = a_{max}\ \eta\ s\ \varphi\ f_b\ f_d \qquad (2)$$

which includes two factors f_b and f_d for build-up and decay, respectively.

With a view towards ^{60}Co typical periods of activation are assumed to be $\tau = t = 0.5$ half-lives, giving $f_b\ f_d = 0.2$, s to be 1.00 and η to vary between 0.1 and 1. The result, *for a nominal neutron flux density of* 10^{14} cm^{-2} s^{-1} ($\varphi = 1$) is presented in Table 4, together with similarly typical data for the previously considered nuclides.

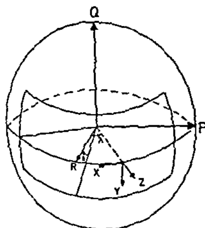


Fig 6 The spherical coordinate system P, Q, R for the definition of the beam channels in space

data for calculation from equations (4) and (5) the absorbed dose in soft tissue having the chemical net formula $(C_5H_{40}O_8N)_n$ and 1 g cm^{-3} density are given in Table 7

The relative biologic efficiency for fast neutrons is between 2 and 5, implying that a dose of 4 to 10 krad would be needed for necrotizing a small tissue volume with neutrons Flux densities of the order of 10^9 cm^{-2} s^{-1} or more are required to deliver the necessary dose in approximately one hour (Table 7) However, existing neutron sources cannot produce well collimated beams with such high fluence rates at present Thus, utilization of a nuclear reactor is technically not realistic, neither is the use of neutrons produced by nuclear reactions induced by alpha emitting nuclides suitable A ^{210}Po-beryllium source, ($T_{\frac{1}{2}} \approx 138$ days), giving neutrons of a mean energy of about 5 MeV, would have to represent an activity of the order of 1 MCi to reach the same dose rate as for the ^{60}Co apparatus in question, ^{252}Cf ($T_{\frac{1}{2}} \approx 1\ 6$ y) emits $2\ 3 \times 10^6$ neutrons/μg s with a mean energy of 2 3 MeV and also $1\ 3 \times 10^7$ photons At present, the cost of a ^{252}Cf source of sufficiently high activity would be tremendous Today, the most favourable neutron source, physically and technically, seems to be the 'D-T generator' It produces 14 MeV neutrons from a tritium target bombarded with low energy deuterons However, the dose rate would be unsatisfactory low even for radiation therapy, approximately 10 rad min Thus, it can be concluded that fast neutrons present no alternative to electro-magnetic radiation in clinical radiation surgery

Relative biologic efficiency

The possibilities of attaining a similar biologic effect with narrow beam techniques based on different types of radiation are dependent both on the

Table 7

Data for dose calculations on narrow neutron beams

Neutron energy Γ (MeV)	Free mean path λ (cm)	Linear attenuation coefficient μ (cm⁻¹)	Coefficient for imparted energy a_Γ (erg cm² g⁻¹)	Relative dose at a depth of 8 cm in tissue	Fluence rate for attaining 10 krad per hour at a depth of 8 cm (cm⁻² s⁻¹)
1.0	2.5	0.40	$0.29 \times 10^{[illegible]}$	0.04	$2.4 \times 10^{[illegible]}$
2.0	4	0.25	$0.35 \times 10^{[illegible]}$	0.14	$5.7 \times 10^{[illegible]}$
5.0	7	0.14	$0.55 \times 10^{[illegible]}$	0.33	$1.5 \times 10^{[illegible]}$
10	9	0.11	$0.63 \times 10^{[illegible]}$	0.41	$1.1 \times 10^{[illegible]}$
15	10	0.10	$0.71 \times 10^{[illegible]}$	0.45	$0.9 \times 10^{[illegible]}$

The energy of the radiation is low, but still high enough to ascertain the demands above of the dose distribution at the centre of the brain. Further, it would allow convenient radiation shielding. ^{46}Sc might also be a possible future alternative.

Fast neutrons, here considered for the energy range 1 to 15 MeV, interact with tissue predominantly by elastic collisions with hydrogen. They have a free mean path λ (2.5 to 10 cm) roughly varying linearly with the energy. In total, the absorption is very complex but approximately the dose distribution along the axis of a narrow neutron beam can, as for the gamma beams (SARBY) be attributed to the first collision, an approximation valid because of the short range of the recoil particles.

The dose rate, $\dot{D}(z)$ erg g⁻¹ s⁻¹ (well approximated by the kerma rate) at a depth of z (cm) in tissue irradiated with a beam containing monoenergetic (E MeV) neutrons of a flux density φ (z) (cm⁻² s⁻¹) is given by

$$\dot{D}(z) = a_\Gamma \ \varphi(z) = a_\Gamma \ \varphi(o) \ e^{-\mu z} \qquad (4)$$

where the attenuation coefficient $\mu = \frac{1}{\lambda}$ (cm⁻¹) and a_Γ is an energy transfer coefficient (erg cm² g⁻¹) given by the expression

$$a_\Gamma = \sum_k \sum_l N_k \ \sigma_{k_l}(E) \ t_{k_l} \ (E) \qquad (5)$$

with N_k the number of atom species K per cm³, σ_{k_l} (E) the cross section for any interaction (l) involving element K, and t_{k_l} (E) the total energy imparted to secondary radiations as a result of this interaction (ROSSI 1956). Relevant

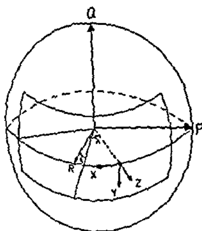


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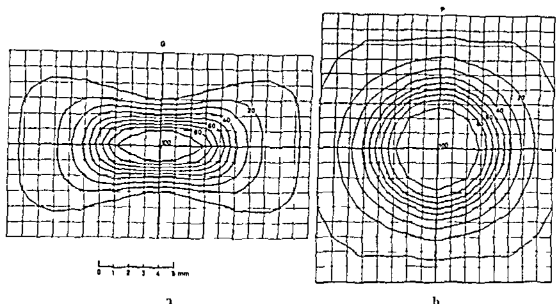


Fig 7 *Calculated distribution of dose as a result of superposed irradiation by 179 beams with a* geometrical cross section of 3 mm × 7 mm all directed towards a common centre of irradiation (the beam focus) The brain is simulated of an isocentric spherical water phantom, 16 cm in diameter The calculations refer to two perpendicular planes a) P = 0 and b) Q = 0 with the beams distributed within $\beta = \pm 35°$ and $\lambda = \pm 80°$ according to the coordinate system defined in Fig 6 The beam geometry *is identical with that chosen in the actual construction (Figs 8, 9) Related to the patient* the dose distributions refer to (a) sagittal plane and (b) frontal plane

macroscopic dose distribution and on the microscopic energy absorption in time and space The biologic efficiency of untested types of radiation can be predicted from a comparison of LET distributions Thus, the possibilities o using ^{60}Co radiation or supervoltage roentgen radiation can be considered on the basis of the conditions for 185 MeV proton radiation as a reference (LARSSON 1962, LARSSON & SARBY 1975) All three types of radiation can be characterized as 'low-LET' radiation and have similar LET distributions (ICRU 1970) This means that after the passage of a primary ionizing particle in a cell the initial spurs with diameters up to 2 nm are well isolated, with a mean separation of 100 nm It is therefore improbable that interaction between diffusing reaction products from one spur to another will influence the biologic efficiency of these radiations

The probability of reactions between spurs from two different primary particles passing through a cell depends on the momentary fluence rate in the beam, which is to be considered relative to the convenient mean dose rate (100 to 1000 rad/min) for the clinical applications If this probability is to be

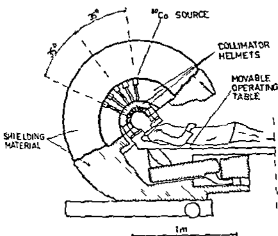


Fig 8 Section through the apparatus with ^{60}Co gamma radiation with patient in position for irradiation Five of the beam channels are shown The diagram refer to the plane P = 0 in Fig 6 (The apparatus was manufactured by AB Motala Verkstad Motala Sweden) The system of collimators (mounted in steel helmets) and steel shields is also indicated

of the same size as the probability for the interspur reactions mentioned above, the mean distance between the primary particles also must be about 100 nm The situation arises when the beam contains 10^{10} particles per cm^2 within a period shorter than the life time of the reaction products ($\ll 10^{-4}$s), which is a condition that cannot be met for a ^{60}Co technique

Accelerators for light ions and roentgen radiation produce radiation in pulses 1 to 10 μs long with intervals of 1 to 100 ms, also implying that the mean dose rate mentioned corresponds to a fluence per pulse of much less than 10^{10} particles per cm^2 Consequently, regarding the time distribution of energy absorption for the discussed types of radiation, differences in their biologic efficiencies are unlikely That these types of radiation have the same biologic efficiency when used for radiation therapy with broad beams has been concluded from a review of a large number of examinations (STENSON 1969)

Design of an apparatus for ^{60}Co gamma radiation

The criteria out lined on p 513 to 514 lead to the following demands on field size, distribution of dose and minimum dose rate in the target area (1) The geometrical beam cross section should be from 3 mm × 5 mm to 3 mm × 12 mm, (2) a dose of approximately 20 krad should be given to the target centre within 3 hours or less, (3) the expected border zone (at the level of the highest dose gradient) between the area of necrosis and, practically, non affected tissue should be as narrow as possible

By accepting ^{60}Co gamma radiation the main lines of a possible construction emanate directly, as the dimensions of the radiation shield and the available

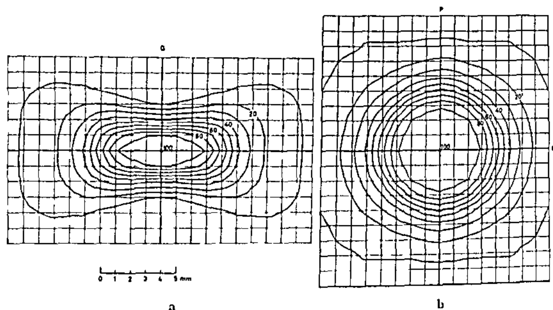


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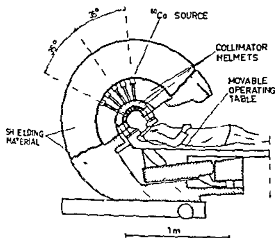


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should be ...

B

cm

Table 8

Secondary radiation doses to various parts of the body at a given dose of 20 000 rad to the centre of the target volume

Part of body	Absorbed dose (rad)
Blood forming organs*	30
Gonads	6
Eye	24
Skull bone	24
Nasopharynx	38
Thyroid	42
Spinal cord	20—40
Lung	30
Kidney	6
Foot	2

* Estimated with respect to the distribution of red bone marrow in an adult person

pecific activity of ^{60}Co set practical limits for the distance between the radiation sources and the target centre The material, some kilocurie of ^{60}Co, has to be divided into many thin needles permitting it to come close to the target without undue penumbra effects, a principle previously suggested for use in radiation therapy by ELLIS & OLIVER (1951) The distance between the sources and the beam focus, i e the point of intersection of the beam axes to which the centre of the target volume is to be positioned stereotaxically, is 38 cm (Fig 8)

The single beam channel Based on these considerations and detailed experiments of the system of beam collimation (SARBY) an attempt was made to optimize the collimation of a narrow gamma beam (Fig 3) with the intention to reduce the possibilities for photons scattered in the walls of the primary collimator to reach the patient Compared with the proton beam previously used for clinical applications the dose gradient in the penumbra region was somewhat more favourable for the gamma beam shaped with this system while the dose contributions outside the penumbra regions were about the same (Figs 4, 5)

However, because of high manufacturing cost for the collimator system (Fig 3), the construction of the treatment apparatus had later to be based on a technical simplification, identical to the alternative C described by SARBY



Fig 9 The inner collimator helmet (Fig 8), in position outside the apparatus seen obliquely from underneath The helmet is mounted at the operating table (Fig 8) The horizontal spindles (coinciding with the P axis in Fig 6) are used for fixation of the stereotaxic instrument

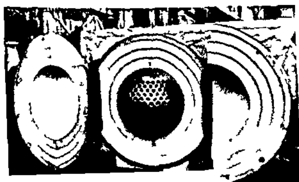


Fig 10 Construction ready for loading with ^{60}Co The system of concentric semi spherical radiation shields is opened from the rear so as to show the position of holders for the source capsules

It gave a slightly higher but still tolerable dose to the brain tissue outside the geometrical penumbra region of the beam

The cross section of the individual beams was (as in Fig 3) defined by sets of exchangeable collimators For practical and economic reasons the collimators consisted of one set of permanent primary collimators and two sets of consecutive beam defining collimators, mounted in spherical steel helmets, the inner being exchangeable so as to permit easy variation of the larger diameter of the cross section (Figs 8, 9)

The beam geometry The number of beam channels is dependent on the total amount of ^{60}Co and its specific activity (Tables 4, 5) All sources are located on a spherical surface, the centre of which coincides with the beam focus (Fig 8) The distribution of the sources of the sphere and the angles of incidence of the single beams determine, at given dimensions of the apparatus, the distribution of the absorbed dose in the brain The positions of the sources may be defined by the latitude angle β and the longitude angle λ in a spherical coordinate system P, Q, R (Fig 6) The equatorial plane $Q = 0$ and the polar axis both form 45° angle against the axis of the patient (Fig 8) The sources are placed symmetrically in regard to the equator and they are almost uniformly distributed over the spherical sector $\lambda = \pm 80°$, $\beta = \pm 35°$

The angle between two neighbouring sources varies between 8 1° and 10° and the corresponding distance is between 5 4 and 6 6 cm This arrangement is dictated by the necessity to work with a large number of sources, presently 179 (the position $\lambda = 0$, $\beta = 0$ is used for accessory mechanical arrangements and a less precise burrhole is not used)

The distribution of the sources is as far as possible adapted to the demand for dose distributions suitable for disc-shaped lesions The theoretically best solution is to distribute the sources as widely as possible within the meredian angular interval and at the same time, let the maximum latitude β_{max} be determined by the conditions of the gradient in the lesion's borderline The choice of $\beta_{max} = 35°$ is aiming at a sufficiently steep dose in the border line of the lesion without adventuring its disc shape

The resulting well-circumscribed, disc-shaped dose distribution (Fig 7) was calculated for the case when all apertures were manufactured for a geometrical field size of 7 mm × 3 mm An alternative dimension 5 mm × 3 mm was also tested clinically (LEKSELL 1971)

It must be clear that this construction with 179 different radiation sources is necessary only because the useful photon-yield per mm² effective source area is so low In fact this yield is about 1000 times lower, than what could be obtained from a well focused electron beam from a linear accelerator Thanks to the separation of the active material, however, the dose gradients may be kept large Nevertheless irradiation could be performed in periods which are not more than a factor of 10 longer than those which would be used at an accelerator (Table 6)

The separation of the active material into many sources permits that the individual beam can be kept at the same position in relation to the object during the whole irradiation The dose accumulated in the radiation field at the surface of the brain during a typical irradiation with 20 krad is then at most about 200 rad, that is a dose which locally applied should be well tolerat-

ed by all intervening tissues, if care is taken to avoid the eye lenses Thus, it is evident that the need for rotation of the object during irradiation is eliminated The result is in fact practically equivalent to one single beam passing over the whole spherical sector covered by the set of cobalt sources The fine structure expected to occur by the many field irradiation is not evident in or near the target area (Fig 7) The parallel is strict also in regard to the relatively unimportant fact that the resulting rotation around two perpendicular axes is not constant but varies by the beam direction

The unit was constructed with high mechanical precision in the collimating systems and in the alignment of the beam axes The definition of the beam focus thus lay within a sphere with a radius of 0 1 mm

Radiation protection The external shielding consists of a spherical steel shell (Figs 8, 10) with a thickness of 35 cm as was concluded from Monte Carlo calculations performed by LEIMDORFER (1963) The fixture for the head of the patient firmly attached to a helmet containing the final collimator set moves with the stretcher in and out through an opening in the shell (Figs 8, 9), that the fixation is stable throughout the irradiation is indicated on the manoeuvre table Visual and verbal contact with the patient can be kept during irradiation

The penetration through the shielding, precalculated and checked by standard survey meters, was in conformity with current regulations for therapy equipments Some secondary radiation emanated through the opening during irradiation, the risks for patients in reproductive age or pregnant patients may be estimated from Table 8 The radiation protection of the body is, even for patients in reproductive age, fully acceptable and seems to be comparable with that at other forms of radiation therapy in the head and neck region

The integral dose to the brain for a central dose of 20 krad and a field of 5 mm × 3 mm was estimated to be about 150 kg rad

Handling of the apparatus The construction permits simplicity in handling which is exceptional compared to other radiation equipments The patient is moved in and out of the apparatus by means of a pneumatic system

A physicist must be responsible for the regular calibration of the dose rate, for the radiation safeness of patients and personnel, and together with the surgeon, for the treatment plan With these precautions, however, the apparatus can be served by the ordinary staff of the clinic and it can be used, after determination of the individual depth dose factor for the mean depth of the target structure in the head, to administer accurate doses by means of a watch and a curve showing the dose rate as a function of the date of radiation

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RÉSUMÉ

Les auteurs examinent les conditions physiques permettant d obtenir de petites lesions en forme de disque pour la chirurgie fonctionnelle par les radiations au moyen d une irradiation par un faisceau étroit Le but etait de mettre au point une technique de traitement pour l usage clinique courant presentant une tres bonne reproductibilite physique et mecanique Les auteurs ont examine les possibilites d utiliser des electrons de haute energie, une irradiation par les rayons roentgen à supervoltage et les isotopes emetteurs gamma ou les neutrons rapides par une technique d utilisation clinique courante Compte tenu des proprietes physiques des facteurs biologiques de la radiation et des circonstances pratiques, le ^{60}Co a ete considere comme la radiation de choix Les auteurs se proposent de realiser un appareil de traitement contenant 179 sources ^{60}Co situees dans un secteur spherique de 70° de latitude et 160° de longitude

REFERENCES

AUSTIN N A Electronic weapon against cancer Electronics 6 (1964) 88

BRAHME A Multiple scattering of relativistic electrons in air Internal Report SSI 1971–043 1971 Institute of Radiation Physics Stockholm

BRYNJOLFSSON A and MARTIN III T Bremsstrahlung production and shielding of static and linear electron accelerators be[illegible]
and radiation protection inst[illegible]

BERLIN T E A general theory [illegible]

DAHLIN H Om optimering av [illegible]
Internal report (GWI R 1/70) Gustaf Werner Institute Uppsala 1970

— and SARBY B Destruction of small intracranial tumours with ^{60}Co gamma radiation Physical and technical considerations To be published in Acta radiol Ther Phys Biol.

ELLIS F and OLIVER R A design for a 2400 curie Co^{60} unit for radiotherapy *In* Radioisotope techniques Vol I p 1 Proceedings of the Isotope Techniques Conference Oxford 1951 H M Stationery Office 1951

ICRU Linear energy transfer International commission on radiation units and measurements Report 16 ICRU Publications Washington 1970

ICRP International Commission on Radiological Protection Protection against ionizing radiation from external sources Publication 15 Pergamon Press, Oxford 1970

JESSEN K Measurements of primary spectra from a kilocurie ^{60}Co unit and a 6 MeV linear accelerator Acta radiol Ther Phys Biol 12 (1973) 561

LARSSON B On the application of a 185 MeV proton beam to experimental cancer therapy and neurosurgery A biophysical study Acta Universitatis Upsaliensis Abstract of Uppsala dissertations in Science No 9 (1962

LEKSELL L and REXED B The use of high energy protons for cerebral surgery in man Acta chir scand. 125 (1963) 1

— and SARBY B Equipment for radiation surgery using narrow 185 MeV proton beams Dosimetry and design To be published in a Suppl to Acta radiol

LAWRENCE J H TOBIAS C A BORN J L WANG C and LINFOOT J A Heavy particle irradiation in neoplastic and neurologic disease J Neurosurg 19 (1962), 717

LEDERER M HOLLANDER J and PERLMAN I Tables of isotopes Sixth edition John Wiley & Sons New York 1967

Conclusions

The great advantage in the use of a nuclide in the present apparatus for cerebral radiation surgery is the absolute reproducibility which the procedure permits. Through the constancy of the decay of the nuclide, the radiation energy and the beam geometry, the dose distribution is fully reproducible from one patient to another. The only physical factor that varies slightly is the dose rate. Through strict control of this parameter, with knowledge of a possible dose rate dependence of the clinical effect, the operation can be given the character of a standard procedure, and that in the best physical meaning of that term.

As the construction is to a great extent based on the experiences with the proton beam at Uppsala it is gratifying to conclude that the dose distributions over a cross section in the region of the lesion and its nearest surroundings are very similar, at the same time as the biologic efficiency also can be expected to be the same.

Acknowledgement

This investigation was supported by the Swedish Cancer Society, the Swedish Medical Research Council and the Axel Axson Johnson Foundation

SUMMARY

The physical conditions for producing minute disc-shaped lesions for functional radiation surgery by means of narrow beam irradiation are discussed The intention was a treatment procedure for routine clinical use and with high physical and mechanical reproducibility The possibilities of using high energy electrons, supervoltage roentgen radiation, gamma emitting nuclides or fast neutrons in a technique for routine clinical use were investigated The radiation of choice taking physical properties, radiation biologic factors and practical circumstances into account was considered to be ^{60}Co gamma radiation A treatment apparatus containing 179 ^{60}Co sources within a spherical sector of 70° latitude and 160° longitude was constructed

ZUSAMMENFASSUNG

Die physikalischen Voraussetzungen, um kleine scheibenförmige Läsionen zur funktionellen Strahlenchirurgie unter Anwendung der Feinstrahl-Bestrahlung hervorzurufen, werden diskutiert Das Ziel war ein Behandlungsverfahren für den klinischen Routinebetrieb und mit hoher physikalischer und mechanischer Reproduzierbarkeit Die Möglichkeiten, hochenergetische Elektronen, hochvolt Röntgenbestrahlung, Gamma-strahlende Radioisotopen oder schnelle Neutronen bei einer Technik für den klinischen Routinegebrauch zu verwenden, wurde untersucht Als die Bestrahlung der Wahl unter Berücksichtigung physikalischer Eigenschaften, radiobiologischer Faktoren und praktischer Gegebenheiten wurde die ^{60}Co Gamma-Strahlung befunden Eine Behandlungsapparatur bestehend aus 179 ^{60}Co Quellen innerhalb eines sphärischen Sektors von 70° Latitude und 160° Longitude wurde konstruiert

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Les auteurs examinent les conditions physiques permettant d'obtenir de petites lésions en forme de disque pour la chirurgie fonctionnelle par les radiations au moyen d'une irradiation par un faisceau étroit Le but était de mettre au point une technique de traitement pour l'usage clinique courant presentant une très bonne reproductibilite physique et mecanique Les auteurs ont examiné les possibilités d'utiliser des electrons de haute energie, une irradiation par les rayons roentgen à supervoltage et les isotopes émetteurs gamma ou les neutrons rapides par une technique d'utilisation clinique courante Compte tenu des proprietes physiques, des facteurs biologiques de la radiation et des circonstances pratiques le ^{60}Co a ete considere comme la radiation de choix Les auteurs se proposent de réaliser un appareil de traitement contenant 179 sources ^{60}Co situees dans un secteur spherique de 70° de latitude et 160° de longitude

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AUSTIN N A Electronic weapon against cancer Electronics 6 (1964), 88

BRAHME A Multiple scattering of relativistic electrons in air Internal Report SSI 1971—043, 1971 Institute of Radiation Physics, Stockholm

BRYNJOLFSSON A and MARTIN III T Bremsstrahlung production and shielding of static and linear electron accelerators below 50 MeV Toxic gas production, required exhaust rates, and radiation protection instrumentation Int J appl Radiat 22 (1971), 29

BURLIN T E A general theory of cavity ionization Brit J Radiol 39 (1966), 727

DAHLIN H Om optimering av strâldosfordelningar vid cerebral strålkirurgi (In Swedish) Internal report (GWI-R 1/70), Gustaf Werner Institute, Uppsala 1970

— and SARBY B Destruction of small intracranial tumours with ^{60}Co gamma radiation Physical and technical considerations To be published in Acta radiol Ther Phys Biol

ELLIS F and OLIVER R A design for a 2400 curie Co^{60} unit for radiotherapy *In* Radioisotope techniques, Vol I p 1 Proceedings of the Isotope Techniques Conference, Oxford 1951 *H M* Stationery Office 1951

ICRU Linear energy transfer International commission on radiation units and measurements Report 16, ICRU Publications, Washington 1970

ICRP, International Commission on Radiological Protection Protection against ionizing radiation from external sources Publication 15 Pergamon Press Oxford 1970

JESSEN K Measurements of primary spectra from a kilocurie ^{60}Co unit and a 6 MeV linear accelerator Acta radiol Ther Phys Biol 12 (1973), 561

LARSSON B On the application of a 185 MeV proton beam to experimental cancer therapy and neurosurgery A biophysical study Acta Universitatis Upsaliensis Abstract of Uppsala dissertations in Science No 9 (1962)

— LEKSELL L and REXED B The use of high energy protons for cerebral surgery in man Acta chir scand 125 (1963), 1

— and SARBY B Equipment for radiation surgery using narrow 185 MeV proton beams Dosimetry and design To be published in a Suppl to Acta radiol

LAWRENCE J H, TOBIAS C A, BORN J L, WANG C and LINFOOT J A Heavy-particle irradiation in neoplastic and neurologic disease J Neurosurg 19 (1962), 717

LEDERER M, HOLLANDER J and PERLMAN I Tables of isotopes Sixth edition John Wiley & Sons, New York 1967

LEIMDORFER M Personal communication 1963
LEKSELL L The stereotaxic method and radiosurgery of the brain Acta chir scand 102 (1951) 316
— Some principles and technical aspects of stereotaxic surgery *In* Pain p 493 Edited by R S Knighton and P R Dumke Little Brown and Company Inc, Boston 1966
— Stereotaxis and radiosurgery An operative system Charles C Thomas Springfield 1971
— HERNER T and LIDÉN K Stereotaxic radiosurgery of the brain Report of a case Kungl fysiograf sällsk Lund forh 25 (1955), 142
LIDÉN K Physikalische Grundlagen für die Verwendung ionisierender Strahlung bei gezielter Hirnchirurgie *In* Handbuch der Neurochirurgie Vol 6 p 199 Edited by H Olivecrona and W Tonnis Springer, Berlin 1957
LINDGREN M Personal communication 1963
ROSSI H Neutrons and mixed radiations *In* Radiation dosimetry, p 667 Edited by G J Hine and G L Brownell Academic Press New York 1956
SARBY B Cerebral radiation surgery with narrow gamma beams Physical experiments Acta radiol Ther Phys Biol 13 (1974), 425
STÉNSON S Effects of high energy protons on healthy organs and malignant tumours Thesis Almqvist and Wiksell Uppsala 1969
TOBIAS C A LAWRENCE J H, LYMAN J BORN J L GOTTSCHALK A LINFOOT J A and McDONALD J Progress report on pituitary irradiation *In* Response of the nervous system to ionizing radiation p 19 Edited by T J Haley and R S Snider Little Brown and Company Boston 1964

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IRRADIATION OF BRAIN METASTASES

H C BERRY, R G PARKER and A J GERDES

CHAO et coll (1954) first published the results of irradiation of brain metastases in 38 patients, 'symptomatic relief' was obtained in 24 (64 %) However, the benefits of palliative therapy for patients with brain metastases have been questioned since these patients often have disseminated disease (CUSHING 1932, LANG & SLATER 1964, STORTEBECKER 1954, TAVERAS 1959) Assessment of palliation has proved to be a most difficult task A surgical criterion for success has been 'increased survival for at least six months without further disability' LANG & SLATER 1964, SIMIONESCU 1960, STORTEBECKER 1954) Amelioration of signs or symptoms has been reported as the criterion for radiation success (CHU & HILARIS 1961, NISCE et coll 1971) However, ultimately it is the functional, subjective or objective improvement of the patient that determines whether a significant palliative result has been achieved

It is the purpose of this report to review the clinical findings, results and implications of the radiation treatment of brain metastases in 124 patients with attention toward palliative results

Submitted for publication 1 April 1974

Table 1

Site of the primary tumor in 124 patients with brain metastases

Primary tumor site	No of patients	Per cent
Bronchus	37	30
Breast	21	17
Unknown	21	17
Genitourinary tract	14	11
Gastrointestinal tract	8	6
Melanoma	7	6
Other	16	13

Materials and Methods

From August 1964 to April 1973, 119 patients with intracranial metastases were accepted for whole brain irradiation An additional five patients received partial brain irradiation The types of primary tumors are presented in Table 1 The largest group of 37 patients (30 %) had bronchogenic carcinoma The second largest groups each containing 21 (17 %) were mammary carcinoma and unknown primary site Eighty-nine (72 %) of the patients had metastases at other sites in addition to having intracranial metastases Seventy-two (58 %) of the primary neoplasms were of a poorly differentiated histologic grade Forty-three (35 %) of the patients were female The intracranial metastases were responsible for the initial sign or symptom of disease in 31 (25 %) of the patients Forty-five (39 %) of the patients had an interval of over 12 months between

Table 2

Presenting signs and symptoms in 124 patients with brain metastases

Symptom and sign	No of patients	Per cent
Cranial nerve deficit	54	44
Headache	52	42
Mental change	51	41
Motor deficit	50	40
Convulsion	31	25
Papilledema	31	25
Visual change	31	25
Cerebellar signs	30	24
Nausea/vomiting	24	19
Sensory deficit	13	10

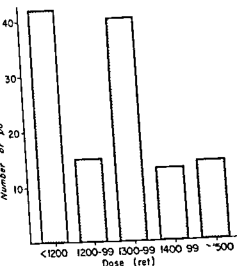


Fig 1 Dose distribution in ret according to Ellis formula

the diagnosis of the primary neoplasm and the diagnosis of intracranial metastasis

The presenting signs and symptoms are listed in Table 2 Cranial nerve deficit, headache, mental change and motor deficit were most common, each being present in about 40 per cent of the patients The diagnosis of intracranial metastasis was established by brain scan in 76 patients (61 %), by arteriography in 25 (20 %), microscopically in 22 (18 %) and on clinical grounds in 29 patients (23 %) Clinically, the intracranial metastases were considered multiple in 53 (44 %), were supratentorial in 82 patients (66 %), subtentorial in 24 patients (19 %) and both sub- and supratentorial in 17 (14 %)

Radiation therapy One hundred and seventeen (96 %) of the patients received supervoltage irradiation (^{60}Co or 8 MeV photons) and in all but five of the 124, the whole brain was irradiated Treatment was incomplete in eight patients and interrupted in two Many different patterns of application were utilized in this retrospective investigation However, the two most commonly used were 3 000 rad/10 Rx/2 weeks or 4 000 rad/20 Rx/4 weeks

According to ELLIS' calculations (1969), 42 (34 %) of the patients received NSD doses of less than 1 200 ret, 15 (12 %) between 1 200 and 1 299 ret, 40 (32 %) between 1 300 and 1 399 ret, 13 (10 %) between 1 400 and 1 499 ret and 14 (11 %) received over 1 500 ret (Fig 1) Sixty-three (51 %) of the patients were on steroids during irradiation and 31 (25 %) were on maintenance chemotherapy Twenty-nine (23 %) patients had a neurosurgical procedure be-

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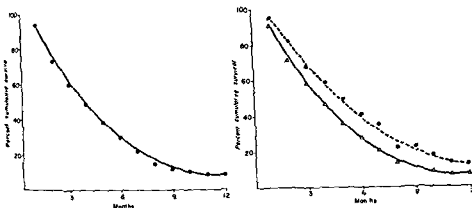


Fig 2 The cumulative survival of 124 patients treated with whole brain irradiation for brain metastases

[illegible]

△ irradiation only

the first day of irradiation, 49 per cent at four months, 30 per cent at six months and 9 per cent at one year The mean survival of our patients was 4 7 months Ten patients survived 13 months or more In the sub-group of 22 patients who had definitive surgery followed by irradiation, the cumulative survival is 68 per cent at three months, 41 per cent at six months and 14 per cent at 12 months respectively (Fig 3) These differences in survival apparently favoring the surgery plus irradiation group over the irradiation only group are never with more than 15 per cent gap and are closer than in those reported by MONTANA et coll (1972) Due to patient selection, these groups are possibly not comparable For example, the patients in the surgery plus irradiation group were more often (90 %) thought to have a solitary intracranial metastasis than were the patients in the irradiation only group (49 %) However, the differences in survival although unidirectional are not statistically significant The overall survivals for the single against multiple metastases groups average 4 7 and 4 8 months, respectively This is also in agreement with MONTANA et coll

In assessment of therapy response, 19 (15 %) patients did not improve with treatment and 20 (16 %) patients deteriorated during irradiation Seventy-nine (63 %) of the patients improved subjectively or objectively and 52 (41 %) improved functionally as measured by the classification of ORDER et coll (1968) The subjective and objective improvements graphically paralleled each other with 41 (55 %) of the 79 improved patients maintaining their subjective or objective improvement through the three-to-six month time interval posttreat-

Table 3

Functional classification of patients with brain metastases

Class	Definitions
I	Intellectually and physically able to work, neurologic findings minor or not present
II	Intellectually intact and physically able to be at home, although nursing care may be required, neurologic finding present but not a major factor
III	Major neurologic findings requiring hospitalization and medical care and supervision
IV	Requires hospitalization and is in serious physical and neurologic state

fore or concomitant with irradiation Eight (6 %) patients were reirradiated when symptoms or signs recurred

The patients were functionally classified before irradiation according to ORDER et coll (1968) (Table 3) The term intellectually intact in Class II means that the patient is oriented and able to engage in ordinary conversation

Improvement is defined as an increase in posttreatment functional status of at least one class over the pretreatment classification The breakdown of patients according to their functional class and treatment received is presented in Table 4 Sixty-three (50 %) of the patients were in Class II before irradiation Surgery indicates attempted resection and not simple needle biopsy or shunt placement

The uncorrected cumulative survival of the entire group of 124 patients is given in Fig 2 The cumulative survival was 60 per cent at three months from

Table 4

Distribution of patients according to functional class and treatment received

Treatment	Class			
	I	II	III	IV
Surgery and irradiation	2	9	10	1
Irradiation	14	54	31	3
Total	16	63	41	4

Table 5

Comparison of both the duration of survival and improvement in patients with functional improvement

Time months	Duration of improvement (%)	Survival (%)	Palliative index improvement/survival (%)
1–3	83	90	92
3–6	52	70	74
6–12	21	37	57
> 1 yr	8	14	57

had *multiple metastases*, the majority of metastases were supratentorial, and the most common presenting signs and symptoms were cranial nerve deficit, headache, mental change and motor deficit, further support previous reports (ASK-UPMARK 1956, BERESFORD 1969, CHAO et coll 1954, CHU & HILARIS 1961, CUSHING 1932, GALLUZZI & PAYNE 1956, GLOBUS & MELTZER 1942, HINDO et coll 1970, LANG & SLATER 1964, MONTANA et coll 1972, NISCE et coll 1971, ORDER et coll 1968, SIMIONESCU 1960, VIETH & ODOM 1965) The fact that 58 per cent of the intracranial metastases were from poorly differentiated primary tumors is not unexpected However, this point has not been emphasized in the recent literature on whole brain irradiation Also, it is of interest that 39 per cent of our patients had an interval of over 12 months between the diagnosis of the primary tumor and the diagnosis of intracranial metastases

The results in terms of length of survival, per cent of functional improvement and palliative index are similar to recent publications (CHU & HILARIS, HINDO et coll, MONTANA et coll, NISCE et coll, ORDER et coll) For the group of patients receiving whole brain doses less than 1 200 ret, the average survival was 4 2 months compared to the group receiving over 1 500 ret whose average was 3 6 months However, this data does not necessarily allow a conclusion that the 1 200 ret level gives overall results equivalent to the 1 500 ret level Six patients received 1 000 rad in a single increment and their average survival was 3 3 months Two were retreated once and one was retreated twice, each time receiving 1 000 rad in a single increment The longest survivor in the single dose group lived eight months and was the individual receiving two retreatments. The (single 1 000 rad) treatments were well tolerated in every instance Steroid coverage was not routinely used in these patients There was no correlation between the use of steroids and survival in the entire group, possibly due to patient selection.

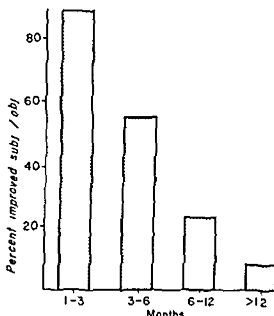


Fig 4 Duration of subjective or objective improvement, post irradiation of 79 patients

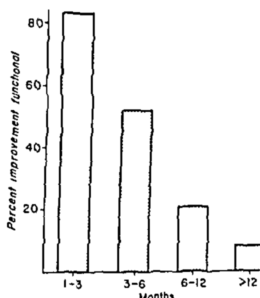


Fig 5 Duration of functional improvement of 51 patients postirradiation

ment Six (8 %) of the 79 improved patients remained improved over 12 months (Fig 4) Twenty-six (52 %) of those 51 patients functionally improved remained in a higher functional status through the three-to-six-month postirradiation period (Fig 5) The mean survival of this group was 6 6 months A ratio of duration of improvement to duration of survival, the 'palliative index' was calculated for this group (ORDER et coll 1968) The index was 92 per cent at one to three months, 74 per cent at three to six months and 57 per cent at six to twelve months (Table 5) In 15 (44 %) of the 34 patients whose brains were examined postmortem, there were multiple cerebral metastases, in 12 (35 %) only a solitary metastasis was demonstrated and in seven patients (21 %), no tumor was identified Four (12 %) patients in this group of 34 had preirradiation histologic proof of intracranial metastasis and none of the seven (21 %) patients without tumor identified at autopsy were biopsed before irradiation

Discussion

The facts that 72 % of our patients had other metastases in addition to intracranial metastases, bronchogenic carcinoma and mammary carcinoma were the most common primary sites, 44 per cent clinically and 47 per cent postmortem

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Average survival of the group of 63 (51 %) on steroids was 4 3 months against 5 0 months for the remainder not receiving steroids These figures would agree with recent publications (HINDO et coll, ORDER et coll) stating that adjunctive therapy with steroids does not seem to significantly influence the palliation rate but may make the critically ill patient more suitable for irradiation

Of the patients receiving concomitant chemotherapy other than steroids, only 5 (4 %) were on agents known to be clinically effective against intracranial metastases All of these patients had either leukemia or lymphoma, had a positive temporal response to whole brain irradiation and would have been expected to respond even without specific chemotherapy Therefore, it cannot be assumed that the results in this group of patients are necessarily due to chemotherapy rather than to irradiation

Regarding additional measures of quality of survival, 63 per cent of the patients noted subjective and objective improvement which was sustained for three to six months in 55 per cent of the group

Of the 7 patients without intracranial metastases at autopsy, all but one had widespread metastatic disease in addition to the diagnosis of intracranial metastases, 2 had positive brain scans and the remaining 5 had various cranial nerve abnormalities with (4) or without (1) other signs or symptoms supporting the clinical diagnosis of intracranial metastases Of this group of 5 clinically diagnosed patients, 3 experienced definite neurologic improvement concomitant with the course of whole brain irradiation, leaving 2 patients in whom the preirradiation diagnosis of intracranial metastases could be seriously raised False positive brain scans, incorrect clinical diagnosis, location miss at the time of autopsy slide sectioning or more hopefully, intracranial tumor eradication secondary to irradiation could explain the lack of tumor in these 7 patients

Patients with untreated intracranial metastases have been shown to have a mean survival of two months and a median survival of one month (LANG & SLATER 1964) Reports of surgical treatment indicate apparent improvement in survival statistics over untreated patients (HAAR & PATTERSON 1972, LANG & SLATER 1964, STORTEBECKER 1954, VIETH & ODOM 1965) However, the postoperative mortality has ranged from 11 to 41 per cent in several reports (HAAR & PATTERSON 1972, LANG & SLATER 1964, RASKIND et coll 1971, RICHARDS & MCKISSECK 1963, SIMIONESCU 1960, STORTEBECKER 1954, VIETH & ODOM 1965) Radiation therapy has not been associated with these high mortality rates

The difference in survival observed in this series between the group of patients treated with surgery plus irradiation and the group treated with irradiation alone is not significant Considering the cost and potential morbidity and mortality associated with the surgical treatment of intracranial metastases, it is felt reasonable to rely on whole brain irradiation alone for the palliation of these patients

SUMMARY

A series of 124 patients with intracranial metastases, treated by irradiation, has been reviewed, retrospectively and prospectively, and the results are reported Worthwhile palliation was obtained in 79 (63 %) of the total number of patients with functional improvement documented in 52 (41 %) The uncorrected mean survival was 4 7 months The addition of surgery to irradiation did not improve results significantly in this series Therefore, surgical removal should be performed on special indications such as rapidly progressing increase in intracranial pressure unresponsive to steroids or shunt, or if the etiology of the intracranial lesion is uncertain

ZUSAMMENFASSUNG

Eine Serie von 124, strahlenbehandelten Patienten mit intrakranialen Metastasen wurde retrospektiv und prospektiv zusammenfassend behandelt und es wird uber die Ergebnisse berichtet Eine wertvolle Palliation wurde bei 79 (63 %) von allen Patienten mit nachweisbarer funktioneller Verbesserung bei 52 (41 %) erzielt Die unkorrigierte mittlere Uberlebenszeit betrug 4,7 Monate Zusätzliche Chirurgie neben der Bestrahlung verbesserte bei dieser Patientenserie nicht signifikant die Ergebnisse Deshalb sollte eine chirurgische Entfernung nur bei besonderen Indikationen wie rasch fortschreitendem gesteigertem intrakraniellem Druck, der nicht auf Steroide oder einen Shunt reagiert, oder bei Unklarheit der Ursache der intrakranialen Schadigung vorgenommen werden

RÉSUMÉ

Les auteurs ont fait une analyse retrospective et prospective d une serie de 124 malades atteints de metastases intra-crâniennes traitees par irradiation et presentent les résultats Ils ont obtenu un resultat palliatif satisfaisant chez 79 malades (63 %) du nombre total de malades avec une amelioration fonctionnelle objective chez 52 malades (41 %) Le taux de survie moyen non corrige était de 4,7 mois L'association d interventions chirurgicales à l irradiation n'a pas amelioré de façon significative les resultats de cette serie C'est pourquoi l exerèse [illegible]
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REFERENCES

ASK-UPMARK E Metastatic tumors of the brain and their localization Acta med scand 154 (1956) 1

BERESFORD H Melanoma of the nervous system Neurology 19 (1969), 59

CHAO J PHILLIPS R and NICKSON J Roentgen-ray therapy of cerebral metastases Cancer 7 (1954), 682

CHU F C H and HILARIS B Value of radiation therapy in the management of intracranial metastases Cancer 14 (1961), 577

CUSHING H Intracranial tumors First edition, p 105 Charles C. Thomas, Springfield,

Illinois 1932

ELLIS F. Dose, time and fractionation: a clinical hypothesis. *Clin. Radiol.* 20 (1969), 1.

GALLUZZI S. and PAYNE P. Brain metastases from primary bronchial carcinoma. Brit. J. Cancer 10 (1956), 408.

GLOBUS J. and MELTZER T. Metastatic tumors of the brain. Arch. Neurol. Psychiat. (Chic.) 48 (1942), 163.

HAAR R. and PATTERSON R. Surgery *for metastatic intracranial neoplasm. Cancer 30 (1972)* 1241.

HINDO W., DETRANA I., LEE M. S. and HENDRICKSON F. Large dose increment irradiation in treatment of cerebral metastases. Cancer 26 (1970), 138.

LANG E. and SLATER J. Metastatic brain tumors. Results of surgical and nonsurgical treatment. Surg. Clin. N. *Amer.* 44 (1964), 865.

MONTANA G., MEACHAM W. and CALDWELL W. Brain irradiation for metastic disease of lung origin. Cancer 29 (1972), 1477.

NISCE L., HILARIS B. and CHU F. C. H. A review of experience with irradiation of brain metastasis. Amer. J. Roentgenol. 111 (1971), 329.

ORDER S., HELLMAN S., VON ESSEN C. and KLIGERMAN M. Improvement in quality of survival following whole brain irradiation for brain metastases. Radiology 91 (1968), 149.

RASKIND R., WEISS S., MANNING J. and WERMUTH R. Survival after surgical excision of single metastatic brain tumors. Amer. J. Roentgenol. 111 (1971), 323.

RICHARDS P. and MCKISSICK W. Intracranial metastases. Brit. J. Med. 1 (1963), 15.

SIMIONESCU M. Metastatic tumors of the brain. A follow up study of 195 patients with neurosurgical considerations. J. Neurosurg. 17 (1960), 361.

STORTI BECKER I. Metastatic tumors of the brain from a neurosurgical point of view: follow-up study of 158 cases. J. Neurosurg. 11 (1954), 84.

TAVERAS J. Radiotherapy of brain tumors. Clin. Neurosurg. 7 (1959), 200.

VIETH R. and ODOM G. Intracranial metastases and their neurosurgical treatment. J. Neurosurg. 23 (1965), 375.

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TABLES FOR USE IN NSD CALCULATIONS

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The concept of Nominal Standard Dose (NSD) is finding increasing use in radiation therapy and while slide rules (WINSTON et coll 1969, RAO 1972) and tables (KROENING & DEITERMAN 1971) have been prepared to aid in the calculation of NSD, the tables presented here do provide greater versatility and ease of use than those previously published The work reported here was done before the report by ORTON & ELLIS (1973) describing the time, dose and fractionation factor (TDF) and represents an alternative approach which is perhaps not so far removed from the original NSD concept

The concept of NSD was recently introduced in the output of a computer program (JONES & WASHINGTON 1973) for external beam calculations and the following is extracted from a manual describing the service It is intended as a guide to the therapist who is already acquainted with original papers describing the NSD concept and its limitations (ELLIS 1968, 1969, 1971, FOWLER 1971 *LIVERSAGE 1971*, *PROBERT 1971*)

The NSD is defined as follows

$$NSD = \frac{TD}{N^{0.24} \times T^{0.11}}$$

Submitted for publication 5 May 1974

Table 1

Three fractions per week

Treat	Mon, Wed, Fri						Mon, Tue, Thu		
Start	Mon		Wed		Fri		Mon		Tue
N	T	$N^{0.76}/T^{0.11}$	T	$N^{0.76}/T^{0.11}$	T	$N^{0.76}/T^{0.11}$	T	$N^{0.76}/T^{0.11}$	T
4	7	2 32	7	2 32	7	2 32	7	2 32	7
5	9	2 67	9	2 67	10	2 64	8	2 70	9
6	11	3 00	12	2 97	12	2 97	10	3 03	13
7	14	3 28	14	3 28	14	3 28	11	3 28	14
8	16	3 58	16	3 58	17	3 56	15	3 61	16
9	18	3 86	19	3 84	19	3 84	17	3 89	20
10	21	4 12	21	4 12	21	4 12	21	4 12	21
11	23	4 38	23	4 38	24	4 36	22	4 40	23
12	25	4 64	26	4 62	26	4 62	24	4 66	27
13	28	4 87	28	4 87	28	4 87	28	4 87	28
14	30	5 11	30	5 11	31	5 09	29	5 13	30
15	32	5 35	33	5 33	33	5 33	31	5 37	34
16	35	5 56	35	5 56	35	5 56	35	5 56	35
17	37	5 79	37	5 79	38	5 77	36	5 81	37
18	39	6 01	40	5 99	40	5 99	38	6 03	41
19	42	6 21	42	6 21	42	6 21	42	6 21	42
20	44	6 43	44	6 43	45	6 41	43	6 44	44
21	46	6 64	47	6 62	47	6 62	45	6 65	48
22	49	6 83	49	6 83	49	6 83	49	6 83	49
23	51	7 03	51	7 03	52	7 02	50	7 05	51
24	53	7 23	54	7 22	54	7 22	52	7 25	55
25	56	7 42	56	7 42	56	7 42	56	7 42	56
26	58	7 61	58	7 61	59	7 60	57	7 62	58
27	60	7 80	61	7 79	61	7 79	59	7 82	62
28	63	7 98	63	7 98	63	7 98	63	7 98	63
29	65	8 17	65	8 17	66	8 15	64	8 18	65
30	67	8 35	68	8 34	68	8 34	66	8 36	69
31	70	8 52	70	8 52	70	8 52	70	8 52	70
32	72	8 70	72	8 70	73	8 69	71	8 72	72
33	74	8 88	75	8 87	75	8 87	73	8 89	76
34	77	9 05	77	9 05	77	9 05	77	9 05	77
35	79	9 22	79	9 22	80	9 21	78	9 23	79
36	81	9 39	82	9 38	82	9 38	80	9 41	83
37	84	9 55	84	9 55	84	9 55	84	9 55	84
38	86	9 72	86	9 72	87	9 71	85	9 74	86
39	88	9 89	89	9 88	89	9 88	87	9 91	90
40	91	10 05	91	10 05	91	10 05	91	10 05	91
41	93	10 21	93	10 21	94	10 20	92	10 23	93
42	95	10 38	96	10 37	96	10 37	94	10 39	97

Table 1 *(cont.)*

	Tue, Thu, Fri								
	Thu		Tue		Thu		Fri		
$N^{0.24}/T^{0.11}$	T	$N^{0.24}/T^{0.11}$	T	$N^{0.24}/T^{0.11}$	T	$N^{0.24}/T^{0.11}$	T	$N^{0.24}/T^{0.11}$	N
2.32	7	2.32	7	2.32	7	2.32	7	2.32	4
2.67	11	2.61	9	2.67	8	2.70	11	2.61	5
2.94	12	2.97	10	3.03	12	2.97	13	2.94	6
3.28	14	3.28	14	3.28	14	3.28	14	3.28	7
3.58	18	3.53	16	3.58	15	3.61	18	3.53	8
3.82	19	3.84	17	3.89	19	3.84	20	3.82	9
4.12	21	4.12	21	4.12	21	4.12	21	4.12	10
4.38	25	4.34	23	4.38	22	4.40	25	4.34	11
4.60	26	4.62	24	4.66	26	4.62	27	4.60	12
4.87	28	4.87	28	4.87	28	4.87	28	4.87	13
5.11	32	5.08	30	5.11	29	5.13	32	5.08	14
5.31	33	5.33	31	5.37	33	5.33	34	5.31	15
5.56	35	5.56	35	5.56	35	5.56	35	5.56	16
5.79	39	5.76	37	5.79	36	5.81	39	5.76	17
5.98	40	5.99	38	6.03	40	5.99	41	5.98	18
6.21	42	6.21	42	6.21	42	6.21	42	6.21	19
6.43	46	6.40	44	6.43	43	6.44	46	6.40	20
6.61	47	6.62	45	6.65	47	6.62	48	6.61	21
6.83	49	6.83	49	6.83	49	6.83	49	6.83	22
7.03	53	7.00	51	7.03	50	7.05	53	7.00	23
7.20	54	7.22	52	7.25	54	7.22	55	7.20	24
7.42	56	7.42	56	7.42	56	7.42	56	7.42	25
7.61	60	7.58	58	7.61	57	7.62	60	7.58	26
7.77	61	7.79	59	7.82	61	7.79	62	7.77	27
7.98	63	7.98	63	7.98	63	7.98	63	7.98	28
8.17	67	8.14	65	8.17	64	8.18	67	8.14	29
8.32	68	8.34	66	8.36	68	8.34	69	8.32	30
8.52	70	8.52	70	8.52	70	8.52	70	8.52	31
8.70	74	8.68	72	8.70	71	8.72	74	8.68	32
8.85	75	8.87	73	8.89	75	8.87	76	8.85	33
9.05	77	9.05	77	9.05	77	9.05	77	9.05	34
9.22	81	9.20	79	9.22	78	9.23	81	9.20	35
9.37	82	9.38	80	9.41	82	9.38	83	9.37	36
9.55	84	9.55	84	9.55	84	9.55	84	9.55	37
9.72	88	9.70	86	9.72	85	9.74	88	9.70	38
9.87	89	9.88	87	9.91	89	9.88	90	9.87	39
10.05	91	10.05	91	10.05	91	10.05	91	10.05	40
10.21	95	10.19	93	10.21	92	10.23	95	10.19	41
10.35	96	10.37	94	10.39	96	10.37	97	10.35	42

Where T D is the total dose
N is the number of fractions
T is the total elapsed days, the first treatment is given on day zero

The unit of NSD is the ret rad equivalent therapy
It is known that

$$T D = d \times N$$

Where d is the dose per fraction

The assumption of equal increments is implicit in these relationships Substituting and rearranging the equation the following is obtained

$$NSD = \frac{T.D}{N} \times \frac{N^{0\ 76}}{T^{0\ 11}} \quad (1)$$

$$\frac{NSD}{d} = \frac{N^{0\ 76}}{T^{0\ 11}} \quad (2)$$

Tables 1 and 2 present the relationship between the number of days (T) that will elapse given a treatment plan for N fractions, starting on a particular day at three and five fractions per week *The ratio of* $N^{0\ 76}$ *to* $T^{0\ 11}$ *also appears* Similar tables have been generated for other fractionation techniques

Determination of the NSD Basic to an understanding of NSD is the fact that this term must be reserved for a treatment that continues to tolerance level and any other solution to equation 1 is simply a number If tolerance has not been achieved then the partial tolerance is obtained by the proportion of the fractions given to the number of fractions required to achieve the NSD at the same fractionation and daily dose The volume of tissue irradiated or the spatial distribution of the dose is not accounted for in the NSD relationship, while it is known that this does affect tolerance Thus, it should be realized that NSD is a specific quantity and is specified in relation to the therapist's philosophy as to what is tolerable and the treatment technique At the outset the therapist has to translate his current practice into NSD terms

Suppose the standard practice at an institution for treating a certain stage of carcinoma of the bladder is to treat to a total dose of 5200 rad in 26 fractions, treating 5 times per week then, from Table 2 and using equation 1, it is seen that

$$NSD = \frac{5\ 200}{26} \times 8\ 05 = 1\ 610 \text{ ret}$$

At some institutions, the standard practice is to give a split course treatment involving a rest and repeat

Suppose that the prescription reads as follows

d rad/day	N fractions	T days
	Rest G days	
d rad/day	N fractions	T days

As a statement a dose of rad per day is delivered for N fractions which at the number of fractions per week used is seen to take T days followed by a rest of G days then a repeat of the therapy given in the first part

If the treatments would continue to the NSD then

$$\frac{\text{NSD}}{d} = \frac{N_t^{0.24}}{T_t^{0.11}}$$

it can be shown that

$$N_t = N\left[1 + \left(\frac{T}{T+G}\right)^{0.11}\right]$$

from these equations the NSD may be calculated

Consider the following example

400 rad/day	6 fractions	at 3 fractions/week
	Rest 31 days	
400 rad/day	6 fractions	at 3 fractions/week

$$N_t = 6\left[1 + \left(\frac{11}{11+31}\right)^{0.11}\right] = 11.17$$

The value of $\left(\frac{11}{11+31}\right)^{0.11}$ is obtained from Table 3

To the nearest integer this is 11 fractions, thus

$$\text{NSD} = 400 \times \frac{11^{0.24}}{23^{0.11}} = 1752 \text{ ret}$$

The value of $\frac{11^{0.24}}{23^{0.11}}$ is obtained from Table 1

By comparison, if no rest were given

400 rad/day	12 fractions	3 F/week

which using Table 1 gives

$$\text{NSD} = 400 \times 4.64 = 1856 \text{ ret}$$

To summarize, before the NSD concept is used routinely in a department, it is first necessary to determine the NSD for the current practice and it is a

Table 2

Five fractions per week

Treat	Mon, Tue, Wed, Thu, Fri									
Start	Mon		Tue		Wed		Thu		Fri	
N	T	$N^{0.24}/T^{0.11}$	T	$N^{0.24}/T^{0.11}$	T	$N^{0.24}/T^{0.11}$	T	$N^{0.24}/T^{0.11}$	T	$N^{0.24}/T^{0.11}$
4	3	2·54	3	2·54	5	2·40	5	2·40	5	2·40
5	4	2·92	6	2·79	6	2·79	6	2·79	6	2·79
6	7	3·15	7	3·15	7	3·15	7	3·15	7	3·15
7	8	3·49	8	3·49	8	3·49	8	3·49	10	3·41
8	9	3·81	9	3·81	9	3·81	11	3·73	11	3·73
9	10	4·12	10	4·12	12	4·04	12	4·04	12	4·04
10	11	4·42	13	4·34	13	4·34	13	4·34	13	4·34
11	14	4·63	14	4·63	14	4·63	14	4·63	14	4·63
12	15	4·91	15	4·91	15	4·91	15	4·91	17	4·84
13	16	5·18	16	5·18	16	5·18	18	5·11	18	5·11
14	17	5·44	17	5·44	19	5·38	19	5·38	19	5·38
15	18	5·70	20	5·63	20	5·63	20	5·63	20	5·63
16	21	5·88	21	5·88	21	5·88	21	5·88	21	5·88
17	22	6·13	22	6·13	22	6·13	22	6·13	24	6·07
18	23	6·37	23	6·37	23	6·37	25	6·31	25	6·31
19	24	6·61	24	6·61	26	6·55	26	6·55	26	6·55
20	25	6·84	27	6·78	27	6·78	27	6·78	27	6·78
21	28	7·01	28	7·01	28	7·01	28	7·01	28	7·01
22	29	7·23	29	7·23	29	7·23	29	7·23	31	7·18
23	30	7·45	30	7·45	30	7·45	32	7·40	32	7·40
24	31	7·67	31	7·67	33	7·62	33	7·62	33	7·62
25	32	7·89	34	7·83	34	7·83	34	7·83	34	7·83
26	35	8·05	35	8·05	35	8·05	35	8·05	35	8·05
27	36	8·25	36	8·25	36	8·25	36	8·25	38	8·20
28	37	8·46	37	8·46	37	8·46	39	8·41	39	8·41
29	38	8·66	38	8·66	40	8·61	40	8·61	40	8·61
30	39	8·86	41	8·81	41	8·81	41	8·81	41	8·81
31	42	9·01	42	9·01	42	9·01	42	9·01	42	9·01
32	43	9·21	43	9·21	43	9·21	43	9·21	45	9·16
33	44	9·40	44	9·40	44	9·40	46	9·36	46	9·36
34	45	9·60	45	9·60	47	9·55	47	9·55	47	9·55
35	46	9·79	48	9·74	48	9·74	48	9·74	48	9·74
36	49	9·93	49	9·93	49	9·93	49	9·93	49	9·93
37	50	10·11	50	10·11	50	10·11	50	10·11	52	10·07
38	51	10·30	51	10·30	51	10·30	53	10·26	53	10·26
39	52	10·48	52	10·48	54	10·44	54	10·44	54	10·44
40	53	10·66	55	10·62	55	10·62	55	10·62	55	10·62

Table 2 *(cont.)*

Treat Mon Tue Wed Thu Fri

Start	Mon		Tue		Wed		Thu		Fri	
N	T	$\frac{N^{0.24}}{T^{0.11}}$	T	$\frac{N^{0.24}}{T^{0.11}}$	T	$\frac{N^{0.24}}{T^{0.11}}$	T	$\frac{N^{0.24}}{T^{0.11}}$	T	$\frac{N^{0.24}}{T^{0.11}}$
41	56	10 80	56	10 80	56	10 80	56	10 80	56	10 80
42	57	10 98	57	10 98	57	10 98	57	10 98	59	10 94
43	58	11 15	58	11 15	58	11 15	60	11 11	60	11 11
44	59	11 33	59	11 33	61	11 29	61	11 29	61	11 29
45	60	11 50	62	11 46	62	11 46	62	11 46	62	11 46
46	63	11 64	63	11 64	63	11 64	63	11 64	63	11 64
47	64	11 81	64	11 81	64	11 81	64	11 81	66	11 77
48	65	11 98	65	11 98	65	11 98	67	11 94	67	11 94
49	66	12 15	66	12 15	68	12 11	68	12 11	68	12 11
50	67	12 31	69	12 27	69	12 27	69	12 27	69	12 27
51	70	12 44	70	12 44	70	12 44	70	12 44	70	12 44
52	71	12 60	71	12 60	71	12 60	71	12 60	73	12 57
53	72	12 77	72	12 77	72	12 77	74	12 73	74	12 73
54	73	12 93	73	12 93	75	12 89	75	12 89	75	12 89
55	74	13 09	76	13 06	76	13 06	76	13 06	76	13 06
56	77	13 22	77	13 22	77	13 22	77	13 22	77	13 22
57	78	13 38	78	13 38	78	13 38	78	13 38	80	13 34
58	79	13 54	79	13 54	79	13 54	81	13 50	81	13 50
59	80	13 69	80	13 69	82	13 66	82	13 66	82	13 66
60	81	13 85	83	13 81	83	13 81	83	13 81	83	13 81
61	84	13 97	84	13 97	84	13 97	84	13 97	84	13 97
62	85	14 12	85	14 12	85	14 12	85	14 12	87	14 09
63	86	14 28	86	14 28	86	14 28	88	14 24	88	14 24
64	87	14 43	87	14 43	89	14 40	89	14 40	89	14 40
65	88	14 59	90	14 55	90	14 55	90	14 55	90	14 55
66	91	14 70	91	14 70	91	14 70	91	14 70	91	14 70
67	92	14 85	92	14 85	92	14 85	92	14 85	94	14 82
68	93	15 00	93	15 00	93	15 00	95	14 97	95	14 97
69	94	15 15	94	15 15	96	15 12	96	15 12	96	15 12
70	95	15 30	97	15 27	97	15 27	97	15 27	97	15 27

mistake to take NSD values quoted in the literature unless the treatment techniques described in the reports are precisely followed

Partial tolerance During the course of a treatment, a calculation can be made to see if tolerance has been reached or possibly exceeded

For example, if NSD is considered 1 770 ret and 25 fractions of 200 rad have been given treating five fractions a week, starting on Monday, from Table 2 it is seen that the number obtained is, using equation 1, $200 \times 7\ 89 = 1\ 578$, which is less than that considered tolerance According to Ellis (1968) this represents a partial tolerance which is defined by the ratio of the number of fractions given to that required to achieve the NSD At the rate specified,

$$\frac{\text{NSD}}{\text{d}} = \frac{1\ 770}{200} = 8\ 85$$

from Table 2 it is seen that this ratio is closely obtained for 30 fractions hence the Partial Tolerance (PT) $= \frac{25}{30} \times 1\ 770 = 1\ 475$ ret

Planning a treatment to give the NSD If the number of fractions is decided then the daily dose may be calculated to give the NSD For example, if treating 5 times a week starting on a Monday, the number of fractions chosen is 38 and NSD for the department is 1 800 ret The daily dose required is, using a rearrangement of equation 2 and Table 2

$$1\ 800/10\ 30 = 174\ 8 \text{ rad}$$

More usually, the dose per fraction is known and the number of fractions required to give the NSD are sought for For example, say the NSD at an institution is considered 1 780 ret and it is planned to give a daily dose of 180 rad five times per week starting on Thursday The ratio of NSD to daily dose is $1\ 780/180 = 9\ 89$ and by inspection of Table 2 the nearest integer number of fractions giving this ratio is 36 fractions in 49 days, which has a ratio of 9 93 The daily dose needs to be modified slightly to achieve the exact NSD and actually $1\ 780/9\ 93 = 179\ 3$ rad should be delivered daily

Changing the prescription The NSD concept may be employed as a guide in changing or modifying the prescription following a gap in the course of a treatment

Consider the following example The prescription of 6 000 rad in 29 fractions over 38 days treating 5 times per week is made, starting on October 16 After 12 fractions, 15 days, for some reason, the patient cannot be treated until Wednesday, November 29th The elapsed days since the start of treatment is 44 days

The partial tolerance achieved by the first part of the treatment is proportioned by the number of fractions

Table 3

Ratio days, $\left(\frac{T}{T+G}\right)^{0.11}$

$\frac{T}{T+G}$	$\left(\frac{T}{T+G}\right)^{0.11}$	$\frac{T}{T+G}$	$\left(\frac{T}{T+G}\right)^{0.11}$	$\frac{T}{T+G}$	$\left(\frac{T}{T+G}\right)^{0.11}$	$\frac{T}{T+G}$	$\left(\frac{T}{T+G}\right)^{0.11}$
0.01	0.603	0.26	0.862	0.51	0.929	0.76	0.970
0.02	0.650	0.27	0.866	0.52	0.931	0.77	0.972
0.03	0.680	0.28	0.869	0.53	0.933	0.78	0.973
0.04	0.702	0.29	0.873	0.54	0.934	0.79	0.974
0.05	0.719	0.30	0.876	0.55	0.936	0.80	0.976
0.06	0.734	0.31	0.879	0.56	0.938	0.81	0.977
0.07	0.746	0.32	0.882	0.57	0.940	0.82	0.978
0.08	0.757	0.33	0.885	0.58	0.942	0.83	0.980
0.09	0.767	0.34	0.888	0.59	0.944	0.84	0.981
0.10	0.776	0.35	0.891	0.60	0.945	0.85	0.982
0.11	0.784	0.36	0.894	0.61	0.947	0.86	0.984
0.12	0.792	0.37	0.896	0.62	0.949	0.87	0.985
0.13	0.799	0.38	0.899	0.63	0.950	0.88	0.986
0.14	0.806	0.39	0.902	0.64	0.952	0.89	0.987
0.15	0.812	0.40	0.904	0.65	0.954	0.90	0.988
0.16	0.817	0.41	0.907	0.66	0.955	0.91	0.990
0.17	0.823	0.42	0.909	0.67	0.957	0.92	0.991
0.18	0.828	0.43	0.911	0.68	0.958	0.93	0.992
0.19	0.833	0.44	0.914	0.69	0.960	0.94	0.993
0.20	0.838	0.45	0.916	0.70	0.962	0.95	0.994
0.21	0.842	0.46	0.918	0.71	0.963	0.96	0.996
0.22	0.847	0.47	0.920	0.72	0.965	0.97	0.997
0.23	0.851	0.48	0.922	0.73	0.966	0.98	0.998
0.24	0.855	0.49	0.925	0.74	0.967	0.99	0.999
0.25	0.859	0.50	0.927	0.75	0.969	1.00	1.000

$$1.792 \times 12/29 = 741 \text{ ret}$$

The partial tolerance will have 'decayed' when treatment is resumed and the partial tolerance on resumption of treatment is obtained from a ratio of the elapsed days,

i.e. Partial Tolerance on resumption $= 741 \times (15/44)^{0.11}$

$$= 658 \text{ ret}$$

Since on November 29th 658 ret have been given, the residual tolerance is

$$1.792 - 658 = 1.134 \text{ ret}$$

It is now decided to complete the course treating about 220 rad per day, three times per week, Monday, Wednesday and Friday, starting on Wednes-

day. It is first necessary to determine how the NSD would be achieved using this fractionation scheme

$$\text{i.e. } 1.792/220 = 8.15$$

From Table 1, it is seen that this is approximated by 29 fractions. To achieve the NSD exactly, the daily dose should be

$$1.792/8.17 = 219 \text{ rad}$$

Since an NSD of 1.792 ret is delivered in 29 fractions a residual tolerance of 1.134 ret is delivered in

$$1.134/1.792 \times 29 = 18.3$$

i.e. 18 fractions to the nearest integer.

In conclusion, this work was prompted by a belief that a parameter describing a radiation therapy is a worthwhile objective and that existing techniques for the calculation of NSD had not gained wide acceptance. The form of the tables presented here do have a marginal advantage in the development of the concept since if the exponents in the relationship are a function of the number of treatments per week as proposed by PROBERT (1971), the tables will be easy to modify.

Acknowledgement

The support of the Board of Directors of the Northwest Medical Physics Center for this work is sincerely appreciated. I am also grateful to Dr Hans Bichsel for assistance in preparing a computer program to generate the tables and Mr Peter Wootton for useful discussion in the *preparation of this paper*.

SUMMARY

The NSD concept has been introduced in the dose calculation service provided by the Northwest Medical Physics Center. Tables have been generated relating the elapsed time to the number of fractions together with the ratio $\frac{N^{0.76}}{T^{0.11}}$. The use of these tables is illustrated by examples.

ZUSAMMENFASSUNG

Das NSD Konzept wurde in den Dosis Berechnungs Service des Northwest Medical Physics Center eingeführt. Es wurden Tabellen aufgestellt, um das Verhältnis der verflossenen Zeit zur Anzahl von Fraktionen zusammen mit dem Verhältnis $\frac{N^{0.76}}{T^{0.11}}$ in Beziehung zu stellen. Die Anwendung dieser Tabellen wird am Beispiel verdeutlicht.

RÉSUMÉ

Le concept de NSD a été introduit dans le service de calcul de dose fourni pour le Centre de Physique Médicale du Northwest Des tables ont été établies reliant le temps écoulé au nombre de fractions et au rapport $\frac{N^{0.24}}{T^{0.11}}$ L'utilisation de ces tables est illustrée par des exemples

REFERENCES

ELLIS F The relationship of biological effects of dose time fractionation factors in radio therapy *In* Current topics in radiation research, Vol IV, p 359 North Holland Publishing Co Amsterdam 1968

— Dose, time and fractionation a clinical hypothesis Clin Radiol 20 (1969), 1

— Nominal standard dose and the ret Brit J Radiol 44 (1971), 100

FOWLER J F Experimental animal results relating to time-dose relationships in radiotherapy and the 'ret' concept Brit J Radiol 44 (1971), 81

JONES D and WASHINGTON J T The quantitative description of a radiation therapy plan Presented at the 15th Annual Meeting of the AAPM San Diego July 29—August 2 1973, abstract in AAPM Quarterly Bulletin 7 (1973), 99

KROENING P M and DEITERMAN L H A table for the normalization of time dose relationships Amer J Roentgenol 112 (1971), 803

LIVERSAGE W E A critical look at the ret Brit J Radiol 44 (1971), 91

ORTON C G and ELLIS F A simplification in the use of the NSD concept in practical radio therapy Brit J Radiol 46 (1973) 529

PROBERT J C Doubts about the nominal standard dose Brit J Radiol 44 (1971) 648

RAO G A radiotherapy dosage calculator Presented at the 3rd International Conference on Medical Physics Gothenburg Sweden 1972

WINSTON B M, ELLIS F and HALL F J The Oxford NSD calculator for clinical use Clin Radiol 20 (1969) 8

day It is first necessary to determine how the NSD would be achieved using this fractionation scheme

$$\text{i e} \quad 1\ 792/220 = 8\ 15$$

From Table 1, it is seen that this is approximated by 29 fractions To achieve the NSD exactly, the daily dose should be

$$1\ 792/8\ 17 = 219 \text{ rad}$$

Since an NSD of 1 792 ret is delivered in 29 fractions a residual tolerance of 1 134 ret is delivered in

$$1\ 134/1\ 792 \times 29 = 18\ 3$$

i e 18 fractions to the nearest integer

In conclusion, this work was prompted by a belief that a parameter describing a radiation therapy is a worthwhile objective and that existing techniques for the calculation of NSD had not gained wide acceptance The form of the tables presented here do have a marginal advantage in the development of the concept since if the exponents in the relationship are a function of the number of treatments per week as proposed by PROBERT (1971), the tables will be easy to modify

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high results in normal urine it allows to recognize a significant increase (2–4times) in deoxycytidine excretion Nevertheless, the authors are conscious of the fact that their judgement in these matters may be wrong and this paper is intended only as an impulse for criticism and improvement, in order to arrive at a set of recommendations for compounds to be measured and methods to be used at nuclear accidents

Methods

General comments

Destruction of delicate compounds present in urine must be avoided Transport of urine, if necessary, should be done in the frozen state, and, before freezing urine should be distributed among three samples of about 15 ml each in order to avoid refreezing after thawing Preliminary experiments indicate that short term freezing affects the enzyme activity only to a minor extent Nevertheless, the enzyme tests should be performed without delay upon receipt of the urine and should be followed by the separation of biogenic amines on Biorex 70 and the extraction of 5 hydroxyindolacetate Moreover, the determination of the biogenic amines should be completed on the day of separation Assay of the various compounds separated on the Biorex 50 column will take more than one day because of the delay due to drying and extraction of paper chromatograms Two or three experienced technicians should be able to accomplish all tests within 3 days, a single technician will require about 5 days

The equipment needed for the determination is that usually available in a biochemical laboratory The spectrophotometer (Cecil UV spectrophotometer) should have a semimicrocell requiring a fill of 1 ml or less A spectrofluorometer is needed for determining dopamine, histamine, spermidine and hippuric acid and is preferable to a filter instrument for the other compounds The data have been obtained with a Turner 430 using 3 ml round cuvettes An analysis of all urinary amino acids including β amino isobutyrate is desirable after a nuclear accident No method has been included, however, since details depend on the analyzer in use in the respective laboratory

A list of the potential indicators, together with normal values as extracted from the literature, are presented in the Table All values are related to excretion of creatinine and represent μmole/mg creatinine (for ions, sulfate and total amino acids), μg mg creatinine for the other compounds and mU/mg creatinine for the enzymes Although such reference to creatinine has its draw backs, it is the only means feasible to obtain reproducible data in animals

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ANALYTIC METHODS FOR BIOCHEMICAL INDICATORS OF RADIATION INJURY

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The development of biochemical indicators of radiation damage has been seriously hampered so far by the lack of pertinent information from irradiated man Moreover, the fortunately rare accidents in nuclear industry have not been fully exploited with respect to analyzing all compounds of interest One reason for this failure has been that adequate analytic methods were not available in the institutions treating the patient

This paper attempts to assemble the analyses which in the opinion of the authors, appear most suitable to assess potential biochemical indicators of radiation damage Several of these methods were developed or modified in this laboratory It should be understood that the choice of substances tested as well as of the methods utilized represents a compromise between the most sensitive and *specific method available and the one which will yield useful* results with a reasonable effort The dilemma may be illustrated for the case of deoxycytidine This substance—an interesting indicator present in rats—is *excreted by man only in minute amounts* (<10 μg/day) A specific and sensitive method for deoxycytidine would require at least three times more work than the one suggested here Although the latter one gives erroneously

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Many compounds are excreted in greater amounts by rat than by man (Table) This is well known for deoxycytidine and creatine but is also marked for taurine, sialic acid, kynurenic acid, amylase and β glucuronidase Moreover, our values for certain compounds (deoxycytidine, kynurenic acid, xanthurenic acid) are significantly larger than those reported in the literature This is probably due to the lower specificity of our methods and could be avoided only at the expense of considerably more work (additional column separation) which would make the techniques impracticable after an accident

Preliminary separations

Preparation of resins Prepare Biorex 50 Wx 8 H^+ 200—400 mesh (Dowex 50) by treating the resin successively with several volumes of 2 N NaOH, H_2O, 2 N HCl and H_2O to neutrality Fill to a height of 7 ml into 10 ml plastic syringes which have a polypropylen filter disk (RCM 1000, Mannesmann) on the bottom and a stopcock at the outlet Wash column with 30 ml H_2O before use

Prepare Biorex AG 1×8 formate 200—400 mesh (Dowex 1) by treating it with 2 N NaOH, H_2O, 2 N formic acid, H_2O Fill into 2 ml plastic syringes to 0 7 ml, wash with 5 ml H_2O, drain

Prepare Biorex 70 Na^+ 100—200 mesh by washing resin with 2 N HCl, H_2O, 2 N NaOH, H_2O, phosphate buffer pH 7 0 (9 25 g KH_2PO_4 2 H_2O, 4 27 g Na_2HPO_4 2 H_2O, 1 [illegible] and adjusted to pH 7 0 Fc [illegible] height of 3 ml and wash w [illegible]

Prepare Biorex AG 1 OH^- by treatment with 2 N NaOH and H_2O Prepare a slurry of Biorex AG 1 OH^- as well as of Biorex 50 H^+ 1/1 with water For methylpyridone carboxamides fill into 2 ml plastic syringes successively 0 1 ml AG 50, 0 4 ml AG 1, 0 2 ml AG 50, 0 5 ml AG 1 and 0 25 ml AG 50 Let resin settle between additions to obtain sharp zones, wash column with 20 ml of water, drain

Separation on Biorex 50 H^+ Spike one 10 ml urine sample with the following standards 2000 μg taurine, 2000 μg indoxylsulfate, 2000 μg pseudouridine, 100 μg xanthurenic acid, 100 μg kynurenic acid, 30 μg *N-methylnicotinamide*, 50 μg delta aminlevulinic acid, 1 μg (in rats 500 μg) deoxycytidine Prepare the other samples by adding to 10 ml urine 0 1 μCi (of pseudouridine ^{14}C and 1 μCi of deoxycytidine ^{3}H Apply the 10 ml to Biorex 50 columns, discard the initial 5 ml of effluent, then collect and wash column with 15 ml of water, yielding *Fraction A (20 ml)* Remove for determinations 2×20 μl for taurine and 2×500 μl (2×100 μl for rats) for indoxylsulfate Apply 200 μl to 2 5 cm

Table

Normal values in human and rat urine

	Man, reported in literature	Man, as determined by methods proposed	Rat as determined by methods proposed
Na^+ (μmole/mg creatine)	100	110	90
K^+	50	40	45
Sulfate, total	20	20	40
Sulfate free	14	15	20
Creatinine (mg/ml)	1.5	1.5	1 (low, due in part to dilution from washing)
Total amino acids (μmole/mg creatinine)	12	15	10
Taurine (μg/mg creatinine)	90	140	300
Creatine	20	10	100
δ aminolevulinic acid	2.0	2.5	2
Hydroxyproline	15	30	80
Hippuric acid	300	300	350
Spermidine	0.65 (total)	0.12 (free)	0.16 (free)
Uric acid	300	440	250
Pseudouridine	80	100	120
Deoxycytidine	0.003	<0.01	50
Xanthurenic acid	1.0	4	4
Kynurenic acid	2.0	5	7
Indoxylsulfate	50	50	250
N methyl nicotinamide	3.5	2.0	3
N methylpyridone carboxamide	15	30	20
Sialic acid	35	80	200
Noradrenalin	0.08	0.07	0.15
Dopamine	0.10	0.20	0.50
Serotonin	0.10	0.15	0.30
5 hydroxyindol acetic acid	1.5	1	1
Histamine	0.02	0.05	0.05
Amylase		0.70	10
RNAase		2	4
Proteolytic activity	20	10	20
β glucuronidase		0.2	2.5

Values for ions sulfate and amino acids are given in μmole/mg creatinine, for other compounds as μg/mg creatinine and for enzymes as mU/mg creatinine

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Individual determinations

All determinations except where indicated otherwise (deoxycytidine) are made in duplicate ΔE signifies that a respective photometric, ΔF that a fluorometric blank is subtracted Δ Est and Δ Fst are the values for the standards indicated for the determinations All calculations yield values in mg/ml urine (or μmole/ml) They must be divided subsequently by the creatinine concentration

Na^+, K^+ *ions* Dilute 0 5 ml of urine with 4 5 ml of water Measure ion activity with specific electrodes (Orion), then spike with 100 μl 1 M KCl and 100 μl 1 M NaCl solution Measure again Calculate by known addition method

Total and free sulfate (standard 50 μl of 1 mg/ml K_2SO_4)

Total sulfate		*Free sulfate*
50 μl urine		50 μl urine
50 μl 2N HCl		50 μl 2N HCl
Hydrolyse in closed tube at 100°C for 4 hours		Continue directly
Add 2 5 ml of 5 % trichloracetic acid, centrifuge, remove		20 μl
1 ml		1 ml
200 μl	reagent	200 μl

Read at 500 nm after 20 min

$$\text{Total (or free) sulfate } \mu\text{mole/ml} = 28\ 7 \frac{\Delta E}{\Delta Est}$$

Reagent Dissolve 0 5 g gelatine Difco in 100 ml warm water, store overnight in refrigerator, dissolve 0 5 g Ba Cl_2 and store for 4 more hours The reagent is stable in the refrigerator for 1 month (DODGSON 1961)

Creatinine (standard 10μl of 1 mg/ml 1 N HCl)

10 μl urine

1 ml alkaline picrate freshly prepared by mixing 5 ml 1 N NaOH and 10 ml 0 6 % picric acid

Read at 530 nm after 20 min standing

$$\text{Creatinine mg/ml} = \frac{\Delta E}{1 Est}$$

Creatine (standards 10 μl of creatine 1 mg/ml, 200 μl of creatinine 1 mg/ml)

The entire procedure is carried out in an ice bath, blanks are made for each urine sample and are measured immediately after addition of the reagents

wide strips of Whatman 3 MM paper Develop by descending chromatography with isopropanol /NH_4OH/H_2O[20/1/2] for 48 hours Localize, the pseudouridine band under ultraviolet light with the help of a reference strip, cut out, concentrate material at one edge by letting water rise 2 times, dry and elute the edge of the paper with 3 ml of H_2O

Wash column with 100 ml of 0 2 N HCl (16 4 ml HCl/l) followed by 10 ml of H_2O and 10 ml of NH_4OH (1 part conc NH_4OH, 4 parts H_2O) Discard these effluents Elute with 10 ml of the NH_4OH solution This eluate should turn alkaline during the first 2 to 3 ml, otherwise the elution volumes must be adjusted accordingly.

Fraction B (10 ml) Remove 4×200 μl for determination of kynurenic and xanthurenic acid Dry rest under hood, redissolve in 500 μl of H_2O fraction B 1, remove 3×20 μl for delta aminolevulinic acid, pass remainder through Biorad AG 1 formate column and wash with 2×0 5 ml of water yielding

Fraction C (1 5 ml) Remove 3×50 μl for N-methylnicotinamide dry rest, dissolve in 300 μl of 50 % methanol, apply to 2 5 cm wide strips of acid washed Whatman 3 MM *paper and develop descendingly in ethyl acetate/* formic acid/H_2O [15/10/15] overnight, localize under ultraviolet light with the help of a reference strip cut out, concentrate at one edge, elute finally with 1 5 ml of 0 0075 N HCl of which 100 μl are used for counting and 700 μl for determination of deoxycytidine In the case of rat, deoxycytidine, present in much greater concentrations can be determined directly in 5 μl of fraction B 1 to which 0 7 ml of 0 0075 N HCl are added Ten μl of fraction C is used for counting radioactivity

Column chromatography of biogenic amines Spike a 10 ml urine sample with 2 μg of histamine, dopamine, noradrenalin and serotonin (200 μl of 10 μg/ml solutions) and with 100 μg of spermidine (100 μl of 1 mg/ml solution) Apply 10 ml urine samples to Biorex 70 columns, wash with 30 ml of water containing 0 1 g EDTA/l pH 7 0, then with 8 ml of 0 2 N HCl containing 0 1 g EDTA/l Elute amines with 10 ml of 0 2 N HCl EDTA Use 3×1 ml for serotonin, 2×1 ml for histamine spermidine Neutralize 2 ml for determination of noradrenalin, dopamine *(see under special methods)* Hydrochloric acid blanks used in these assays must also pass the Biorex 70 column

Chromatography of N-methyl pyridone carboxamide on layered column Apply 750 μl urine and wash with 2 ml water, collect and pass again through column Then wash with 3 ml water, collect total eluate and drain Prepare also a column for blank

	(136 g Na acetate 57 ml acet acid/l) heat 10 min to 95°C	
	acetylacetone	10 μl
500 μl	Ehrlich reagent (1 g dimethylaminobenzaldehyde in 34 ml acetic acid, add 16 ml $HClO_4$ conc)	500 μl

Read at 546 nm after 15 min

$$\text{Delta amino levulinic acid } \mu g/ml = 2\ 72 \times \frac{\Delta E}{\Delta Est}$$

(Sun et coll 1969)

Total hydroxyproline (standard 10 μl of 100 μg/ml)
100 μl urine
100 μl HCl conc
Hydrolyse at 100° in closed tubes for 18 hours
Neutralize with 1 N NaOH, adjust volume to 4 ml
500 μl sample
300 μl chloramin T freshly prepared solution
(1 41 g chloramin T/10 ml buffer pH 6 0 (50 g citric acid 12 ml acetic acid 120 g Na acetat 34 g NaOH 1250 ml H_2O and 250 ml n propanol)
keep 20 min at 20°C add 300 μl Ehrlich reagent freshly prepared (1 5 g di methylaminobenzaldehyde) in 7 4 ml n propanol, add slowly 2 6 ml conc perchloric acid)
Heat to 60° C for 15 min
Read at 555 nm

$$\text{Hydroxyproline } \mu g/ml = 80 \frac{\Delta E}{\Delta Est}$$

(Stegemann & Stalder 1967)

Hippuric acid (standard 5 μl of 500 μg/ml)
5 μl urine
3 ml H_2SO_4 70 % (3 parts H_2O add 7 parts H_2SO_4 Suprapur conc)
Use quartz cuvettes
Read in spectrofluorometer excitation at 260 nm, emission 375 nm

$$\text{Hippuric acid } \mu g/ml = \frac{\Delta F \times 500}{\Delta Fst}$$

(Ellman et coll 1961)

Both sets of standards, creatine and creatinine, must be run simultaneously
50 μl urine (for rat use 10 μl)
1 ml freshly prepared mixture of 30 mg of α-naphthol in 10 ml of 0 5 N NaOH to which 1 5 ml of 0 04 % diacetyl in H_2O is added
read at 530 nm the blank immediately, the assay after 30 min

$$\text{Creatine (man) } \mu g/ml = \frac{200}{\Delta E st\text{-}in}\left[\Delta E - \left(\frac{\text{Concinin} \times \text{Estinin}}{4}\right)\right]$$

$$\text{Creatinine (rat) } \mu g/ml = \frac{1000}{\Delta E st\text{-}in}\left[\Delta E - \left(\frac{\text{Concinin} \times \text{Estinin}}{20}\right)\right]$$

Estin and Estinin are the standards for creatine and creatinine and Concinin the concentration of creatinine in mg/ml obtained for creatine in the test above (GERBER et coll 1961)

Total amino acids and taurine (standards 10 μl of 1 mg/ml taurine, 10 μl of 1 mg/ml alanine or 112 nmole)

Total amino acids		*Taurine*
5 μl urine		20 μl fraction A
1 ml	Ninhydrine freshly mixed	1 ml

Heat to 95°C for 20 min, cool and dilute with 4 ml of 50 % ethanol
Read at 570 nm

$$\text{Taurine } \mu g/ml = 1000 \frac{\Delta E}{\Delta E st\text{-}taur}$$

$$\text{Total amino acids } \mu mole/ml = 22\ 4 \frac{\Delta E}{\Delta E st\text{-}Al}$$

Ninhydrine 25 ml 3 % ninhydrine in methylcellosolve
25 ml Citrate buffer (42 g citric acid, 22 4 g NaOH/l) pH 5 7
200 μl $SnCl_2$ (4 51 g $SnCl_2$ dissolved in 1 7 ml conc HCl add 8 3 ml H_2O) (SORBO 1961)

Delta amino levulinic acid (standard 10 μl of 100 μg/ml)

Sample		*Blank*
20 μl	fraction B	20 μl
10 μl	acetylacetone	0
500 μl	buffer pH 4 6	500 μl
	acetate buffer pH 4 6	

$$\text{Deoxycytidine (rat)} = 0.2 \frac{\Delta E \times \text{Act added}}{\Delta Est \times \text{Act sample}}$$

(CHEN et coll 1968)

Kynurenic acid, Xanthurenic acid (standard 10 μl of 100 μg/ml)

Kynurenic acid,		*Xanthurenic acid*
200 μl fraction B		200 μl fraction B
1 8 ml H_2O		1 3 ml H_2O
cool in ice 1 ml H_2SO_4 conc Suprapur		1 5 ml 40 % NaOH
Read in fluorometer after 30 min		
Excitation	345 nm	345 nm
Emission	440 nm	420 nm

$$\text{Kynurenic (Xanthurenic acid)}\ \mu g/ml = \frac{\Delta F \times 5}{\Delta Fst}$$

(PRICE et coll 1965)

N Methyl nicotinamide (standard 10 μl of 100 μg/ml)
50 μl fraction C
50 μl methylethylketone Mn Cl_2 (2 ml/l methylethylketone of 0 1 M (1 25 %) $MnCl_2$ solution)
50 μl 5N NaOH
shake, wait 10 min
50 μl 6 N HCl prepare blanks without methylethylketone
heat 10 min to 95° C add
2 5 ml 2 5 % KH_2PO_4

Spectrofluorometer	Excitation	360 nm
	Emission	450 nm

$$\text{N Methyl nicotinamide}\ \mu g/ml = \frac{\Delta F \times 3.7}{\Delta Fst}$$

(PRICE et coll)

Indoxylsulfate (Indicane) (standard 10 μl of 1 mg/ml)
500 μl fraction A (rat 100 μl)
500 μl Ehrlich reagent freshly prepared (0 89 dimethylaminobenzaldehyde dissolved in 30 ml methanol+30 ml HCl conc)
20 min
read at 480 mμ

Pseudouridine

1 2 ml eluate | 1 2 ml eluate (from paperchromatogram)

100 μl 1N HCl | 100 μl 1N NaOH

Read L at 290 nm

Calculate $\Delta L = E290$ alk $- E290$ acid

Count 300 μl by liquid scintillation counting

$$\text{Pseudouridine } \mu g/ml = 0\ 00068 \times \Delta L \times \frac{\text{Act added}}{\text{Act sample}}$$

Uric acid

Use kit marketed by Boehringer (Germany)
50 μl urine
8 ml borate buffer pH 9 5 (sol 1)
mix remove for

Assay		*Blank*
3 5 ml	urine dilution	3 5 ml
20 μl	uricase (sol 2)	0
0	50 % glycerol (sol 3)	20 μl

Mix, measure after 10 min at 293 nm

Uric acid μg/ml $= \Delta E \times 2\ 15$

Deoxycytidine

700 μl in 0 0075 N HCl (eluate from paper (man) or diluted fraction (rat)
heat to 95°C for 2 hours, add
100 μl of 0 025 M periodate in 0 125 N H_2SO_4 (535 mg Na periodate 150 μl H_2SO_4 conc/100 ml H_2O)
20 min at room temperature
200 μl Na arsenite 2 % in 0 5 N HCl
400 μl Thiobarbituric acid (0 75 g in 100 ml H_2O add 700 μl 1 N NaOH)
heat to 95° C for 20 min
cool, extract with 1 2 ml cyclohexanone, centrifuge
read at *532* and *562* nm
$\Delta E = E\ 532 - E\ 562$

$$\text{Deoxycytidine (man) } \mu g/ml \text{ urine} = 0\ 0143 \frac{\Delta E \times \text{Act added}}{1 E st \times \text{Act sample}}$$

Deoxycytidine (rat) $= 0.2 \frac{\Delta E \times \text{Act added}}{\Delta Est \times \text{Act sample}}$

(CHEN et coll 1968)

Kynurenic acid, Xanthurenic acid (standard 10 μl of 100 μg/ml)

Kynurenic acid,	*Xanthurenic acid*
200 μl fraction B	200 μl fraction B
1 8 ml H_2O	1 3 ml H_2O
cool in ice 1 ml H_2SO_4 conc Suprapur	1 5 ml 40 % NaOH

Read in fluorometer after 30 min

Excitation	345 nm	345 nm
Emission	440 nm	420 nm

Kynurenic (Xanthurenic acid) μg/ml $= \frac{\Delta F \times 5}{\Delta Fst}$

(PRICE et coll 1965)

N Methyl nicotinamide (standard 10 μl of 100 μg/ml)
50 μl fraction C
50 μl methylethylketone Mn Cl_2 (2 ml/l methylethylketone of 0 1 M (1 25 %) $MnCl_2$ solution)
50 μl 5N NaOH
shake, wait 10 min
50 μl 6 N HCl prepare blanks without methylethylketone
heat 10 min to 95° C add
2 5 ml 2 5 % KH_2PO_4

Spectrofluorometer	Excitation	360 nm
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(PRICE et coll)

Indoxylsulfate (Indicane) (standard 10 μl of 1 mg/ml)
500 μl fraction A (rat 100 μl)
500 μl Ehrlich reagent freshly prepared (0 89 dimethylaminobenzaldehyde dissolved in 30 ml methanol + 30 ml HCl conc)
20 min
read at 480 mμ

$$\text{Indoxylsulfate (man) } \mu g/ml = \frac{40\ \Delta E}{\Delta Est}$$

$$\text{Indoxylsulfate (rat) } \mu g/ml = \frac{200\ \Delta E}{\Delta Est}$$

(RYLANCE 1969).

N-Methyl-2-pyridone 5 carboxamide
Read effluent from column at 310 nm
Compare with effluent of blank column
N-Methyl-2-pyridone-5-carboxamide $\mu g/ml = 0\ 389 \times \Delta E310$
(PRICE et coll)

Sialic Acid total (standard 10 μl of 100 $\mu g/ml$)
50 μl urine
50 μl H_2SO_4 0 5 N (1 part H_2SO_4 conc /64 7 parts H_2O)
heat 1 hour at 80° C, add
100 μl 0 2M perjodate (8 556 g in 200 ml 4 5 M H_3PO_4 (1 part conc /3 37 parts H_2O)
20 min at 20° C then add
500 μl arsenite (10 % Na arsenite in 0 5M Na_2SO_4 (71 g/l Na_2SO_4) plus 60 ml/l H_2SO_4 conc)
shake until brown color disappears (heat slightly if necessary) add 1 5 ml thiobarbituric acid (0 6 % in Na_2SO_4 0 5M (71 g/l))
heat to 95° for 10 min, cool, add
2 ml cyclohexanone, shake, centrifuge
read E of upper layer at 545 nm (wash cuvettes before and after with methanol)

$$\text{Total sialic acid } \mu g/ml = \frac{\Delta E \times 20}{\Delta Est}$$

(WARREN 1959)

Noradrenalin/Dopamine (standard 200 μl of 10 $\mu g/ml$ diluted in 10 ml 0 2 N HCl EDTA and neutralized as described below)
2 ml eluate from Biorex 70 column
1 ml phosphate buffer pH 7 0 (4 27 g Na_2HPO_4 2 H_2O, 9 25 g KH_2PO_4 0 2 g EDTA/200 ml This is the same buffer as used for preparation of resin but 5 times concentrated
200 μl 1N NaOH to adjust pH at 6 5 to 7, check with pH meter

500 μl H_2O to adjust volume to 4 ml, then divide into
2 ml samples
200 μl iodine solution (2 g KI 0 5 g iodine /40 ml H_2O)
mix well and wait exactly 2 min
500 μl freshly prepared sulfite (1 ml 25 % Na_2SO_3 plus 9 ml 5N NaOH)
wait 2 min then add
400 μl conc acetic acid
heat to 45°C for 3 min
Read noradrenalin in spectrofluorometer
excitation 400 nm
emission 485 nm
heat again to 85°C for 30 min
Read dopamine in spectrofluorometer using quartz cuvettes
excitation 330 nm
emission 400 nm

$$\text{Noradrenalin Dopamine } \mu g/ml - \frac{0\ 2\ \Delta F}{\Delta F st}$$

(CHANG 1964)

Histamine Spermidine (free) (standards 200 μl of 10 μg/ml histamine, 100 μl of 1 mg/ml spermidine diluted separately in 10 ml 0 2 N HCl EDTA)
1 ml eluate from Biorex 70 column
500 μl 5 N NaOH
100 μl 0 1 % o phtaldialdehyde in methanol
mix well wait 4 min, add
800 μl 4M H_3PO_4 (28 ml conc H_3PO_4 plus 72 ml H_2O)
read in spectrofluorometer after 30 min
excitation 360 nm
emission 405 and 500 nm

$$\text{Histamine } \mu g/ml - 0\ 2 \times \frac{(\Delta F_{500} \times \Delta F_{sp405}) - (\Delta F_{405} \times \Delta F_{sp500})}{(\Delta F_{H500} \times \Delta F_{sp405}) - (\Delta F_{H405} \times \Delta F_{sp500})}$$

$$\text{Spermidine } \mu g/ml = 10 \times \frac{(\Delta F_{405} \times \Delta F_{H500}) - (\Delta F_{500} \times \Delta F_{H405})}{(\Delta F_{H500} \times \Delta F_{sp405}) - (\Delta F_{H405} \times \Delta F_{sp500})}$$

where F405 F500 are the readings of the sample at the two wavelengths after substraction of blank fluorescence
F_{sp405} F_{sp500} the readings of the spermidine standard
F_{H500} F_{H405} the readings of the histamine standard
(OATES et coll 1962)

Serotonin (standard 200 μl of 10 μg/ml diluted with 10 ml 0 2 HCl EDTA)

Sample (2 ×)		*Blank* (1 ×)
1 6 ml	HCl conc	1 6 ml
100 μl	cysteine 1 % in 0 1 N HCl	
	$NaIO_4$ 0 02 % in 0 1 N HCl	100 μl
1 ml	eluate Biorex 70	1 ml
Mix, incubate at 20°C 30 min		
100 μl	$NaIO_4$	Ø
O	cysteine	100 μl
100 μl	0 1 % o phthalaldehyde in methanol	100 μl

mix, heat to 95°C for 15 min, cool
read in spectrofluorometer excitation 360 nm
emission 480 nm

$$\text{Serotonin } \mu g/ml - \frac{0\ 2\ \Delta F}{\Delta F st}$$

(Maickel et coll 1969)

5 hydroxyindole acetic acid (standard direct 10 μl and extraction 50 μl of 50 μg/ml respectively)
300 μl urine
300 μl H_2O
0 6 g NaCl
50 μl 4 % cysteine
180 μl HCl conc
12 ml diethylether
extract by shaking for 1 min Remove from upper phase
9 ml ether, add
4 ml phosphate buffer pH 7 0 (3 54 g KH_2PO_4 $2H_2O$ 7 24 g Na_2HPO_4 $2H_2O$) shake 1 min centrifuge, remove 3 samples of 1 ml
proceed for determination as described for serotonin replacing eluate with the phosphate buffer extract

$$5\text{ OH indoleacetate } \mu g/ml - 8\ 33 \frac{\Delta F}{\Delta F st\ extern} \text{ or } \frac{8\ 88\ \Delta F}{\Delta F st\ direct}$$

(Korf & Valkenburgh Sikkema 1969)

Proteolytic activity (cathepsin D E C 3 4 4 23)
50 μl urine

200 μl formate buffer 0 2 N pH 3 5 (11 2 ml formic acid/500 ml adjusted with NaOH to pH 3 5 and filled up to 1 l)
500 μl hemoglobin (2 5 % in 0 003N H Cl) (prepared from erythrocytes)
Incubate at 45°C for 1 hour add
1 ml 5 % trichloracetic acid,
centrifuge, remove from supernatant
500 μl, add
300 μl Folin reagent (Folin, Merck, diluted 1/3 with H_2O)
1 ml 1 N NaOH
read after 10 min at 600 nm
prepare also blank by adding TCA before incubation
Standard 10 nmole tyrosine (10 μl of 182 μg/ml)
1 mU = liberation of 1 nmole tyrosine/min/ml urine

Proteolytic activity mU/ml $= 21 \times \frac{\Delta E}{Est}$

(Barrett 1967)

Amylase (E C 3 2 1 1) (Use Rapidstat Kit from Pierce Chem Co) (Standard 10 μl of 2 mg/ml solution of glucose)

Test	*Blank*
10 μl urine (diluted rats 1/10 with saline)	10 μl urine
100 μl substrate	100 μl color reagent
Incubate 15 min at 37°C	
100 μl color reagent	100 μl substrate
Heat 10 min to 95°C	
Cool 1 min, add	
800 μl H_2O	800 μl H_2O

read at 500 nm
1 mU = liberation of μmole glucose/min/ml urine

Amylase (man) mU/ml $= 0\ 747 \times \frac{\Delta E}{Est}$

Amylase (rat) mU/ml $= 7\ 47 \times \frac{\Delta E}{Est}$

Ribonuclease (E C 2 7 7 16)
20 μl urine
400 μl buffer substrate freshly prepared by mixing 2 parts Tris buffer 0 1M

pH 7.4 (12.1 g Tris 10 ml 1 N HCl/l) and 1 part 0.8 % RNA dialyzed previously for 3 hours against distilled water.
Incubate at 37°C for 1 hour, prepare also blanks without incubation and without substrate, add
200 μl uranylacetate 0.75 % in 4.17 M perchloric acid (1 part $HClO_4$ conc., 2 parts H_2O)
centrifuge, remove
200 μl supernatant, dilute with
5 ml H_2O

read E at 260 nm $\quad 1\ mU = \frac{\Delta E_{260}}{min\ ml}$

RNA ase U/ml = 0.0125 × ΔE
(KALNITZKY et coll. 1959).

β-Glucuronidase
50 μl urine
100 μl buffer substrate (10.32 mg phenolphthalein glucuronide in 10 ml acetate buffer pH 4.5 (3.4 g Na acetate, 1.54 ml acetic acid /500 ml)
incubate at 37°C for 6 hours (man) or 1 hour (rat) add
1 ml glycine NaOH (18 g glycine 1 N NaOH to pH 10.5/l)
Read at 546 nm. Prepare also a few blanks without incubation
Glucuronidase (man) mU/ml = 0.0025 × ΔE (1 mU = 1 μmole phenolphtaleine liberated/min)
Glucuronidase (rat) mU/ml = 0.0151 × ΔE
(FISHMAN 1965).

SUMMARY

Since the development of biochemical indicators of radiation injury has been hampered often by the lack of simple and rapid methods of determination, techniques for the measurement of 30 urinary compounds are presented. These methods have been in part taken from the literature, in part they have been modified or developed by us. They represent a compromise between speed and simplicity of execution and specificity of assay.

ZUSAMMENFASSUNG

Die Entwicklung biochemischer Indikatoren des Strahlenschadens leidet unter dem Fehlen einfacher und rascher Bestimmungsmethoden. In dieser Arbeit sind solche Methoden für 30 verschiedene, in den Urin ausgeschiedene Stoffe zusammengefasst. Sie wurden teils der Literatur entnommen, teils in unserm Labor modifiziert oder entwickelt. Hierbei wurde nach einem Kompromiss zwischen Einfachheit und Schnelligkeit der Durchführung und Spezifität der Analyse gesucht.

RÉSUMÉ

Vu que développement des indicateurs biochimiques de lésions des radiations a souvent été entravé par le manque de méthodes de détermination simples et rapides, des techniques de mesures de 30 composés urinaires sont présentées Ces méthodes ont été puisées en partie dans la littérature que nous avons modifiées ou développées Elles représentent un compromis entre la rapidité, la simplicité de l'exécution et la spécificité de l analyse

REFERENCES

Barrett A J Lysosomal acid proteinase of rabbit liver Biochem J 104 (1967), 601

Chang C G A sensitive method for spectrophotofluorometric assay of catecholamines Int J Neuropharmacol 3 (1964), 643

Chen I-Wen Kereiakes J G, Friedman B I and Saenger E. L. Colorimetric analysis of deoxycytidine in urine after separation by ion exchange column Anal Biochem. 23 (1968) 230

Dodgson K S Determination of inorganic sulphate in studies on the enzymic and non enzymic hydrolysis of carbohydrate and the sulphate esters Biochem. J 78 (1961), 312

Ellman G L, Burkhalter A and La Dou J J A fluorometric method for the determination of hippuric acid Lab Clin Med 57 (1961) 813

Fishman W H Determination of β glucuronidases Meth Biochem Anal 15 (1965), 77

Gerber G B Gerber G and Altman K I A method for the isolation and determination of creatine and glycocyamine Anal Chem 33 (1961), 832

Kalnitzky G Hummel J P Resnick H Carter J R, Barnett L. B and Dierks J The relation of structure to enzymatic activity in ribonuclease Ann N Y Acad Sci 81 (1959), 542

Korf J and Valkenburgh Sikkema T Fluorometric determination of 5 hydroxyindolacetic acid in human urine and cerebrospinal fluid Clin chim Acta 26 (1969), 301

Maickel R P Cox R H Saillant J and Miller F P A method for the determination of serotonin and norepinephrin in discrete brain areas Int J Neuropharmacol 7 (1969), 275

Oates J E Mars E and Sjoerdsma A Studies on histamine in human urine using a fluorometric method of assay Clin chim. Acta 7 (1962), 488

Price J M Brown R R and Yess N Testing the functional capacity of the tryptophan-niacin pathway in man by analysis of urinary metabolites Advanc metab Disord 2 (1965) 159

Rylance H G The estimation of indoxylsulfate in urine Clin chim Acta 26 (1969), 99

Sorbo B A method for the determination of taurine in urine Clin chim. Acta 61 (1961), 87.

Stegemann H and Stalder K Determination of hydroxyproline Clin chim. Acta 18 (1967), 267

Sun M W Stein E and Gruen F W A single column method for the determination of urinary δ aminolevulinic acid Clin Chem 15 (1969), 183

Warren L The thiobarbituric acid method for sialic acid J Biol Chem. 234 (1959), 197.

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